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TABLE OF CONTENTS

A method for producing artificial epidemics of Diplodia ear rot. ARNOLD J. ULLSTRUP 93

A comparison of gross pathogenic effects of Pythium graminicola, Pythium debaryanum, and Helminthosporium sativum on seedlings of crested wheatgrass ..... W. F. BUCHHOLTZ 102

Transmission of sugar-beet yellow-net virus by the green peach aphid ..... EDWARD S. SYLVESTER 117

Variability of the cucurbit root-rot fungus, Fusarium (Hypomyces) solani f. cucurbitae ..... N. PRASAD 133

The occurrence of Puccinia coronata on Rhamnus Frangula in Canada ..... B. PETURSON 142

Synchytrium found on the roots of strawberry. S. M. ZELLER AND LEO CAMPBELL 149

Application of the bacteriophage-lysis technique for the identification of plant pathogenic bacteria. H. H. THORNBERRY, A. C. BRAUN, AND R. P. ELROD 152

Chemical soil treatment for black root rot of tobacco in the greenhouse ..... ROBERT A. STEINBERG AND E. E. CLAYTON 155

Sclerotinia trifoliorum, a pathogen of Ladino clover. K. W. KREITLOW 158

Pythium root rot of aroids and Easter lilies. W. B. TISDALE AND GEORGE D. RUEHLE 167

Phytopathological Notes 171

The rôle of thiamin and certain related compounds in pigment production by Corynebacterium michiganense (Erw. Smith) Jensen ..... ARMIN C. BRAUN

A new method of evaluating fungicides ..... J. L. FORSBERG

Rhizoctonia neck and bulb rot of iris in the Pacific Northwest. CLARENCE H. SONDERMAN AND NEIL ALLEN MACLEAN

A METHOD FOR PRODUCING ARTIFICIAL EPIDEMICS OF DIPLODIA EAR ROT[1]

ARNOLD J. ULLSTRUP[2]

(Accepted for publication July 15, 1948)

Diplodia ear rot is one of the major diseases of corn (*Zea mays* L.) in many areas where the crop is grown. Wide differences exist among inbred lines in resistance to the disease, but no line is known to be immune from attack. The only feasible means of controlling this disease is by breeding and selecting for resistance. Because of fluctuation in the prevalence of diplodia ear rot from year to year and from one locality to another in a given season it is not often convenient to rely on natural epidemics to determine the relative resistance of host material.

A satisfactory method of producing artificial epidemics of diplodia ear rot could be a useful adjunct to any corn breeding program where resistance to this is to be incorporated into inbred lines.

The present paper reports the development of an inoculation technique that has proved satisfactory over several years.

MATERIALS AND METHODS

Inoculum was increased on sterile, whole oats in 2-quart fruit jars. The jars were filled to about one-half their capacity with oats and an excess of warm tap water added. After 4 hours the water was drained off and 200 ml. of hot tap water added to each jar. The jars were plugged, pre-heated for 15 minutes, and then autoclaved for 3 hours at 15-lb. pressure.

Isolates of *Diplodia zeae* (Schw.) Lev. were selected on the basis of rapid and abundant sporulation as determined from preliminary tests on sterile whole oats in small flasks or as indicated by direct plating of infected kernels on acidified potato-dextrose agar. Except in an experiment where the virulence of different cultures was compared, only one culture was used for a given experiment. Inocula from different isolates were never mixed to make up a composite spore suspension. The same isolate was not always used from year to year.

The sterile oats were seeded with mycelium of the selected isolate and incubated for about 6 weeks in the light at a temperature ranging between 24° and 26° C. The stock spore suspension was prepared by removing the material from the jars to a cheesecloth sack and kneading the mass in water in order to separate the spores from the oats. The suspension was then

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture; and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station. Journal Paper No. 334 of the Purdue University Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases.

filtered through a layer of cheesecloth and made up to volume at a rate of a jar of inoculum in one quart of water. This stock spore suspension from a good sporulating culture (Fig. 1) was very dark and comparable in color to fresh India ink diluted 1 : 10,000.

The stock suspension was diluted usually at a rate of 2 qt. in 2.5 gal. just prior to spraying on the ears. A simple 2.5-gal. compressed air sprayer was used to apply the spore suspension over the entire ear from

FIG. 1. Fruit jar culture of *Diplodia zeae* after 5 weeks' incubation. The dark areas are composed of masses of pycnidia and pycnospores.

silk to shank. Two and one-half gallons of spore suspension were sufficient to inoculate 250 to 300 plants. Plants were usually inoculated only once, although in some years and in certain experiments two inoculations were made with an interval of 5 to 7 days between applications.

Single cross hybrids were used in all experiments. The relative resistance of an inbred line was determined by comparing the mean amount of disease sustained in all its crosses with the mean amount of disease in any other group of hybrids, within the all-combination set, having another

inbred parent in common. This could be done where inbred lines to be tested were crossed in all possible single cross combinations and compared in a two-way table.

The plots were uniformly 1×10 hills with 3 plants per hill and were replicated 3 to 6 times in a simple randomized block design. Harvests were made usually during the first two weeks in November. Each plot was harvested separately and every ear was inspected in the laboratory. If no disease was observed on the surface, several kernels were removed from points well distributed over the ear to determine the extent of infection, if any, at the kernel tips.

The amount of disease in each plot was expressed as the percentage of *Diplodia*-infected ears; or the percentage by weight of *Diplodia*-infected kernels in a representative 250-gram sample of the shelled corn from an entire plot; or by a disease index calculated by the following formula:

$$\frac{20\ (nC_0 + nC_1 + nC_2 + nC_3 + nC_4)}{N}$$

where n = the number of ears in each class; C_0, C_1, C_2, C_3, C_4 = the class values (0 to 4) for healthy, one-fourth rotted, one-half rotted, three-fourths rotted, and completely rotted ears, respectively; N = the total number of ears in an entire plot. The disease index measured the amount of disease, and weight was given to the extent of rotted area on each ear.

EXPERIMENTAL RESULTS

Influence of time of inoculation. Inoculations were made at the time of full silk or within a week thereafter and continued at weekly intervals for 4 or 5 weeks. A single plot was inoculated only once. Both resistant and susceptible single cross hybrids were used. Inoculum was made up periodically to insure that spores were approximately the same age for all inoculations. The hybrids and different dates of inoculation were randomized independently within each replication. All plots had been planted on the same date.

The greatest amount of infection and the clearest differential among hybrids were secured in those plots inoculated within a week or two after full silk. There was a slight, but non-significant, trend toward a decrease in disease from the first inoculation to that made 3 weeks after full silk. Inoculations made 4 or 5 weeks after full silk resulted in a small amount of ear rot. An example of the difference in percentage of rotted ears sustained from early as compared with late inoculations is shown in table 1.

Effect of dilution of inoculum on the incidence of ear rot. A stock spore suspension, made up at a ratio of one 2-qt. jar of culture per quart of water, was used at rates of 0.5, 1.0, 2.0, and 3.0 qt. stock suspension in 2.5 gal. water. Hybrids and different concentrations of inoculum were randomized independently within each replication.

Even the lowest dilution of 0.5 qt. stock suspension per 2.5 gal. of final

TABLE 1.—*Effect of time of inoculation on the incidence of diplodia ear rot. Early inoculations made about 1 week after full silk*

Single crosses	Time of inoculation	Mean percentage of *Diplodia*-rotted ears	Minimum significant difference	
			1 per cent level	5 per cent level
			Per cent	*Per cent*
Tr × Hy	Aug. 8, 1942	46.2	11.2	7.2
do.	Sept. 5, 1942	23.7		
Hy × 38–11	Aug. 11, 1942	24.7	11.9	7.6
do.	Sept. 8, 1942	7.8		
Wf9 × R4	Aug. 6, 1942	11.6	10.7	6.8
do.	Sept. 5, 1942	2.4		
Tr × Hy	Aug. 7, 1944	77.4	23.0	14.7
do.	Sept. 4, 1944	48.2		
M14 × Hy	Aug. 7, 1944	50.8	25.8	16.5
do.	Sept. 4, 1944	9.5		
540 × 90	Aug. 7, 1944	41.7	15.0	9.5
do.	Sept. 4, 1944	4.3		
R4 × 90	Aug. 7, 1944	26.3	21.1	13.4
do.	Sept. 4, 1944	5.9		

inoculum was sufficient to give a good differential between hybrids (Table 2). Significant differences in percentage of rotted ears are shown between certain dilutions of inoculum when applied to the more susceptible single crosses. The difference between dilutions, however, is not consistently maintained in the resistant hybrids.

Comparison of different isolates of Diplodia zeae. Isolations of *Diplodia zeae* were made from 5 collections of diseased ears gathered from widely separated geographical locations. Each of the 5 isolates was seeded separately on sterile whole oats in the usual manner. Four single crosses were used as host testers, two of which were known to be susceptible and two were known to be relatively resistant. Ears were inoculated 10 days after full silk and again 7 days later. Isolates and hybrids were randomized independently within each replication. Particular care was taken to prevent any mixing of inoculum.

TABLE 2.—*Effect of dilution of inoculum on the incidence of Diplodia-rotted ears*

Single crosses	Mean percentage of *Diplodia*-rotted ears after inoculation with indicated stock spore suspension in 2.5 gal. final inoculum				Minimum significant difference between dilutions	
	0.5 qt.	1.0 qt.	2.0 qt.	3.0 qt.	1 per cent level	5 per cent level
					Pct.	*Pct.*
Tr × M14	56.3	65.5	58.8	69.5	13.7	7.6
RR98 × M14	40.9	47.4	48.0	66.1	5.1	2.8
RR98 × R4	2.1	3.8	0.8	5.4	7.3	4.0
540 × R4	2.1	3.7	1.8	3.2	5.0	2.7
Minimum significant difference between hybrids:						
1 per cent level	3.0	4.4	8.7	6.2		
5 per cent level	1.7	2.5	4.8	3.4		

TABLE 3.—*Incidence of ear rot induced by different isolates of Diplodia zeae. Each single cross inoculated twice with a given culture*

Single crosses	Mean percentage of Diplodia-rotted ears from cultures					Minimum significant difference	
	No. 2	No. 6	No. 9	No. 13	No. 23	1 per cent level	5 per cent level
						Pct.	*Pct.*
Tr × M14	78.2	92.2	91.1	83.6	84.3	13.3	9.5
Tr × Hy	71.8	89.8	85.1	84.5	71.4	12.2	8.7
R4 × 90	17.3	35.2	38.0	13.4	16.1	21.3	15.2
Wf9 × R4	15.6	31.0	29.0	20.7	17.4	13.2	9.4

There was no indication of differential virulence among the cultures with the host materials used (Table 3). Plots inoculated with cultures No. 6 and No. 9 had somewhat more disease than those inoculated with the other cultures. The reason is not known, although there may have been somewhat better sporulation or a higher percentage of viability of the spores.

Indexing of inbred lines. Inbred lines of corn were indexed for resistance to diplodia ear rot for several years within groups combined in systematic single crosses. The object was to determine both the relative resistance of the lines to the disease and the uniformity of disease reaction from year to year. The results of these tests are shown in tables 4 and 5. The inbred lines Tr and M14 have usually contributed high susceptibility to crosses. In some years M14 has had greater susceptibility than Tr but in other years the opposite was true. None of the other inbred lines tested has contributed so much susceptibility to crosses. The inbred lines 540, R4, and 90 invariably have been the lines that contributed the most resistance to their crosses. The inbred lines 38–11, Hy, and Wf9 usually have been of intermediate rank in contributing susceptibility to their crosses.

TABLE 4.—*Mean percentages of Diplodia-rotted ears in 45 hybrids involving 10 inbred lines in all possible single cross combinations. Inoculations made in 1943*

	M14	Tr	A	Wf9	Hy	38–11	66	R4	90	540	Means for inbreds
M14		56.5	79.2	46.5	34.0	36.2	29.1	26.4	24.6	27.0	41.1
Tr	56.5		29.6	42.0	42.4	30.7	30.9	20.9	21.1	9.4	31.5
A	79.2	29.6		17.1	6.3	24.4	21.3	20.0	10.0	5.1	23.7
Wf9	46.5	42.0	17.1		11.5	11.5	15.5	1.9	12.7	6.8	18.4
Hy	34.0	42.4	6.3	11.5		17.9	6.7	24.1	11.2	10.8	18.3
38–11	36.2	30.7	24.4	11.5	17.9		7.1	11.7	10.4	9.4	17.7
66	39.1	30.9	21.3	15.5	6.7	7.1		9.4	6.7	5.3	15.8
R4	26.4	20.9	20.0	1.9	24.1	11.7	9.4		11.1	6.8	14.7
90	24.6	21.1	10.0	12.7	11.2	10.4	6.7	11.1		3.3	12.3
540	27.0	9.4	5.1	6.8	10.8	9.4	5.3	6.8	3.3		9.3

Minimum significant difference for inbred means:
4.8 per cent at 1 per cent level.
3.6 per cent at 5 per cent level.

TABLE 5.—*Mean percentages of Diplodia-rotted ears in 28 hybrids involving 8 inbred lines in all possible single cross combinations. Inoculations made in 1947*

	M14	Tr	38–11	Wf9	Hy	R4	540	90	Means for inbreds
M14		86.5	77.1	78.1	77.9	72.1	71.0	32.8	70.8
Tr	86.5		84.9	91.8	69.1	42.9	59.4	58.6	70.5
38–11	77.1	84.9		78.2	41.0	35.2	24.2	43.9	54.9
Wf9	78.1	91.8	78.2		32.2	14.6	35.2	24.6	50.7
Hy	77.9	69.1	41.0	32.2		43.6	31.6	30.4	46.5
R4	72.1	42.9	35.2	14.6	43.6		6.4	28.3	34.7
540	71.0	59.4	24.2	35.2	31.6	6.4		13.9	34.5
90	32.8	58.6	43.9	24.6	30.4	28.3	13.9		33.2

Minimum significant difference for inbred means:
6.6 per cent at 1 per cent level.
5.0 per cent at 5 per cent level.

Methods of recording data. The amount of ear rot in each plot was recorded as: (1) percentage of *Diplodia*-rotted ears; (2) percentage by weight of *Diplodia*-rotted kernels in a representative 250-gm. sample of the shelled corn; and (3) the disease index. In a number of experiments, two of the three records were used. The coefficients of correlation between these records were calculated and are shown in table 6. The extremely high values for r in different years and when different host materials were used suggests that the three methods of recording disease incidence are equally satisfactory.

DISCUSSION

The success and usefulness of a method for inoculating corn ears with *Diplodia zeae* depend on several criteria: (1) a clear differentiation between resistant and susceptible host materials; (2) disease reactions of inbred lines and hybrids comparable to those experienced under natural epidemics of the disease; (3) reproducibility of results from year to year, and no great effect of environment on the relative disease reactions of the host plants; (4) simplicity of execution and freedom from complicated and laborious procedures; and (5) simulation of those phenomena attending the natural mode of infection by the parasite. *D. zeae* is not a wound para-

TABLE 6.—*Correlations between percentage by weight of Diplodia-rotted kernels and percentage of Diplodia-rotted ears and between Diplodia-rotted kernels and disease index. Correlations based on the mean values for single crosses*

Year	Number of single crosses observed	Number of replications on which means values are based	Variables correlated	Value of r
1941	15	3	Rotted kernels *vs* rotted ears	0.99
1942	15	6	do. *vs* do.	0.99
1943	21	5	do. *vs* do.	0.95
1943	21	5	do. *vs* disease index	0.98

site, and therefore it is conceivable that wounding could nullify effects of barriers, either structural or physiological, that function under normal conditions to impede the entrance of the fungus into the host. Young[3] has described an inoculation method in which toothpicks, over which mycelium of the parasite had been allowed to grow, are inserted into the ears or stalks of corn. Good differentiation between resistant and susceptible host materials are claimed for this method. The inoculation technique described in the present paper amends natural epidemics only in providing for a greater volume and more uniform distribution of inoculum.

The most critical factor involved in the described method is the large scale production of an adequate supply of spores for inoculation. Experiments on dilution of inoculum have shown that a comparatively wide range of spore concentration can be used to obtain a high level of infection. If a poorly sporulating culture is used, the concentration of spores may be too low to obtain sufficient infection for differentiation. Preliminary tests of the sporulating ability of isolates provides for selection of prolific spore producers. A satisfactory culture, when incubated in the light for 4 to 6 weeks at temperatures ranging between 24° and 26° C., produces a large number of spores with a high percentage of germination.

In experiments reported above, the best differential infections were obtained when inoculations were made within a period extending from a few days after full silk until about 2 weeks later. Inoculations made 4 weeks after full silk or later have invariably resulted in appreciably less ear rot. The lower incidence of ear rot following later inoculations may be due to environmental conditions in the late summer unfavorable to the development of diplodia ear rot. Another possible explanation is that there is a shorter period for disease development between inoculation and harvest. The fact that late inoculations have uniformly resulted in less disease even in years when temperatures and humidities were ideal for infection casts some doubt on the possibility that meteorological conditions are responsible for this difference in disease incidence. The possibility that a shortening of the period for disease development accounts for less ear rot following late inoculations is refuted by the observation of rotted ears within two weeks after early inoculations, which is considerably less time than that elapsing between late inoculations and harvest. The most logical explanation of the reduction in ear rot following late inoculations is that the ears become more resistant as they approach maturity. If a block of one single-cross could be inoculated at one time when different plots ranged in stage of development from full silk to 5 weeks after, the question could be solved more precisely. A number of practical difficulties are involved in making plantings at different dates in the spring in order to obtain a situation where such wide stages of maturity exist in an experimental block of one single-cross at the time of inoculation.

[3] Young, H. C. Jr. The toothpick method of inoculating corn for ear and stalk rots. (Abstr.) Phytopath. 33: 16. 1943.

Throughout these experiments, single-crosses have been used and the contribution of the component inbred lines to resistance or susceptibility determined by arranging the data on two-way tables. It is possible that inbred lines, as such, could be used, but confounding factors such as lack of good "fill" of the ears, frequently found in inbred lines, greater susceptibility to other ear diseases, smaller ears, and generally reduced vigor of the entire plant would be encountered.

The amount of diplodia ear rot in each inoculated plot was recorded as percentage of *Diplodia*-infected ears; as percentage by weight of *Diplodia*-infected kernels in a representative 250-gm. sample of shelled corn; or as a disease index. The results of these three methods have been shown to be so closely correlated that the simplest method, namely, that in which the percentage of *Diplodia*-rotted ears is recorded, appears to be adequate. Hoppe and Holbert[4] did not find high correlation coefficients between percentage of rotted ears and percentage by weight of rotted kernels. These authors, however, included all ear rots under conditions of natural infection where the level of disease incidence was appreciably lower than in the experiments reported in the present paper. The exceptionally high correlation coefficients reported here may be due in part to the high incidence of disease and in part to the fact that many ears had incipient infections that were not apparent on the surface. The fact that mean values were used rather than the values of individual plots would also increase the magnitudes of the coefficients of correlation.

A satisfactory method of inoculating corn ears with *Diplodia zeae* could be applied to corn improvement in several ways: (1) Relative resistance of inbred lines to diplodia ear rot could be determined. (2) Heavy epidemics of the disease in segregating populations would permit selection for resistance to diplodia ear rot. (3) Studies of the inheritance of resistance or the nature of resistance to this disease would be facilitated.

SUMMARY

A method for inoculating corn ears with *Diplodia zeae* for differentiating reaction to diplodia ear rot is described.

Large volumes of inoculum were produced on moist sterile whole oats incubated in the light for 4 to 6 weeks at 24° to 26° C. It is important to select isolates that sporulate abundantly and rapidly. Spores for the stock spore suspension were separated from the oat substrate by placing the entire culture from a fruit jar in a cheesecloth sack and kneading the mass in water. The suspension was filtered through cheesecloth and made up to a standard volume at the rate of one jar of culture per quart of water. The stock spore suspension was diluted prior to inoculation at a rate of 2 qt. made up to 2.5 gal. with water. The inoculum was sprayed on the ears, from silk to shank, with a compressed air sprayer of 2.5-gal. capacity.

[4] Hoppe, Paul E. and James Holbert. Methods used in the determination of relative amounts of ear rot in dent corn. Jour. Amer. Soc. Agron. 28: 810–819. 1936.

Two and one-half gallons of spray were sufficient to inoculate 250 to 300 ears.

A high incidence of infection and clear differential effects were obtained when ears were inoculated during a period from a few days after full silk until about two weeks later. Inoculations made 4 or 5 weeks after full silk invariably resulted in a small amount of ear rot.

The stock spore suspension diluted at rates ranging from 0.5 qt. to 3 qt. in 2.5 gal. of final inoculum gave good infection and differential effects.

Experiments with five isolates of *Diplodia zeae,* obtained from ears collected in widely separated geographical areas, failed to demonstrate instances of differential virulence, although some isolates appeared to be somewhat more virulent than others.

Three methods were used to record the incidence of ear rot in inoculated plots. The results of all three methods were very closely correlated. The simplest method is that in which the percentage of rotted ears is recorded.

The inoculation technique gave consistent results when used to index inbred lines for disease reaction. The relative resistance of inbred lines was determined from the results of inoculating all-combination sets of single crosses.

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A COMPARISON OF GROSS PATHOGENIC EFFECTS OF PYTHIUM GRAMINICOLA, PYTHIUM DEBARYANUM, AND HELMINTHOSPORIUM SATIVUM ON SEEDLINGS OF CRESTED WHEATGRASS[1]

W. F. Buchholtz

(Accepted for publication September 5, 1948)

As a means of conserving and building soil, farmers in central South Dakota attempt to seed cultivated land to grass. In recent years, considerable effort has been expended to establish stands of crested wheatgrass (*Agropyron cristatum* (L.) Beauv.). Such attempts have met more often with failure than with success. From 1936 to 1941, for instance, 153,000 acres were seeded to grass under direct supervision of the Soil Conservation Service. Fully half this acreage failed so that reseeding was necessary; in many fields as many as 4 or 5 successive seedings failed, even with approved cultural practices.

It was evident immediately that in many cases, soil on which field plantings of grass had failed would not produce a stand of healthy grass seedlings in the greenhouse, regardless of the moisture supply, and that similar soil, when steamed, yielded excellent stands of vigorous grass seedlings.[2]

There is limited information regarding the nature of forage grass seedling and root troubles in the northern Great Plains. Sprague (4) reported recovery of *Pythium arrhenomanes* Drechsler, *Helminthosporium sativum* P. K. and B., and *Fusarium spp.* from the roots of wheat and a number of forage grasses in the Dakotas. *H. sativum* and *Fusarium spp.* were taken from diseased grass seedling roots collected in eastern South Dakota. Later Sprague and Atkinson (5) reported the susceptibility of 18 species of grasses and cereals to isolates of *P. arrhenomanes* taken from all the species. Recently, Fischer, Sprague, Johnson, and Hardison (3) listed 42 grass species as hosts of *P. arrhenomanes,* 39 species as hosts of *Pythium debaryanum* Hesse, 53 as hosts of *H. sativum,* 36 as hosts of *Rhizoctonia solani* Kuhn, and many species as hosts of several species of *Fusarium.*

Still more recently, Sprague (6, 7) pointed out that *Pythium arrhenomanes* or *P. graminicola* Subr. is the principal cause of seedling blight of grasses during May and June, although seed rotting and interruption of germination can be the result of the activity of *P. debaryanum, P. irregulare* Buis., *Helminthosporium sativum,* and *Fusarium scirpi* var. *acuminatum* (Ell. and Ev.). Sprague's observations are essentially in accord with the information recorded in this paper, which was previously pre-

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series Paper No. 195.

[2] Franzke, C. J. Unpublished data.

sented in abstract form (2). The observations of Andrews (1) also are in essential agreement with Sprague's.

In the investigations here recorded, the symptoms of blighted seedlings are described and the evidence for regarding *Pythium graminicola* as the causal agent is presented. The pathogens most commonly recovered from roots of blighted grass seedlings were *P. graminicola, P. debaryanum, Helminthosporium sativum,* and *Fusarium spp.* The gross effects of these pathogens on seedlings in steamed and clean field soil indicate that *P. graminicola* is the most virulent, and in pure culture most nearly reproduces the symptoms evident on seedlings in infested field soil.

SYMPTOMS OF SEEDLING BLIGHT OF CRESTED WHEATGRASS

Two distinct symptom complexes occur after crested wheatgrass seeds have been planted in many field soils; (1) some seeds are rotted during early stages of germination, and (2) many of the seedlings wilt and die or become partially defoliated after they are a week or ten days old. There is only occasional deterioration of seedlings less than a week old.

Examination of swollen but otherwise nongerminated seeds reveals a reduction of all but the glumes to a soft watery mass. Rarely this rot involves radicles and epicotyls during early stages of germination.

A very few young seedlings may "damp-off" before the first leaf is fully elongated and expanded. A very light brown discoloration and general softening of the axis in a region extending usually upward from the scutellar node is evident on such plants. The tip of the first leaf may lose its green color and quickly become yellow and desiccated. On an occasional plant, the first leaf may break over at the tip of the coleoptile.

The majority of seeds planted in field soil germinate normally and the resulting seedlings appear normal for from 1 to 3 weeks after germination, depending on their rate of growth as determined usually by soil temperature. In the greenhouse, low temperatures may delay growth of the seedlings and also the onset of blight; at higher temperatures seedling growth is more rapid, but the first symptoms usually appear sooner and the final loss of stand is greater. In either case, usually within 2 weeks after completion of germination, the first leaves of infected seedlings begin to wilt (Fig. 5, A). Loss of leaf turgor results in the lack of luster and a consequent lead green color common to wilted leaves. Recovery by individual leaves from this wilted condition seldom occurs, even temporarily. Instead there is gradually increased wilting, followed by loss of green color and finally yellowing and death of the entire leaf.

Sometimes the plant's existence terminates with the death of the first leaf, but frequently the growing point survives to feebly produce a whorl of somewhat dwarfed leaves, the lower ones of which then suffer the same fate. These symptoms suggest failure of water supply to the plant and under stringent moisture conditions might be termed "firing." Actually,

they frequently occur on seedlings in wet soil. Eventually many plants succumb and die, sometimes 6 weeks or more after completion of seed germination.

The roots of afflicted seedlings may exhibit a range of brown discolorations and disintegration. Seedlings that die quickly usually have less than an inch remaining of their primary and seminal roots and these may be brown and softened, particularly at the ends. Plants a month old or older give evidence of having lost roots continuously, with development of new roots on living portions of older ones, commonly above lesions. If a plant lives to tiller, it produces sturdy adventitious roots at the crown which tend to be less disintegrated than the primary and seminal roots. Even so, the watery disintegration of several crowns one-eighth inch in diameter has been observed.

Initial symptoms on the very small primary and seminal roots have not been observed. Wherever infection occurs (apparently near the root tip) the tissues evidently become reddish-brown very quickly. Several attempts to observe pathogens in the cells and tissues or roots of very young seedlings have failed.

While the disease may be much worse in some fields or areas than in others, it apparently does not commonly occur in distinct patches, nor is it restricted to individual plants. Since crested wheatgrass field seedlings are not made under clean culture, but rather under conditions favoring a temporary cover of annual weeds, the mass appearance of an afflicted stand in the field cannot be observed.

Altogether, somewhat more than 400 root fragments of diseased seedlings grown in various field soils in the greenhouse were placed on plain agar (without added nutrients) during the winters of 1940–'41 and 1941–'42. From these were observed to develop: *Pythium graminicola,* 158 times; *Fusarium spp.,* 81 times; *P. debaryanum,* 71 times; *Helminthosporium sativum,* 6 times; and *Rhizoctonia spp.,* twice. All of these fungi except *Rhizoctonia* have been recovered from crested wheatgrass seedlings grown in the field.

GROSS EFFECTS ON CRESTED WHEATGRASS OF SEEDLING PATHOGENS IN PURE CULTURE IN STEAMED SOIL

In order to evaluate and delineate the roles of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* as seedling pathogens of crested wheatgrass, plantings were made in soil infested at various depths below the seed with these 3 pathogens. Also, seedlings of various ages up to 4 weeks were exposed, in steamed soil, to these pathogens. The results of such exposures indicate the pathogenicity of *P. graminicola* at all depths of infestation and to plants of all ages included in the experiments.

In preliminary experiments, in which potato-dextrose-agar cultures of *Pythium graminicola, P. debaryanum, Helminthosporium sativum,* and

Fusarium spp. were placed in contact with the seed in steamed soil, all markedly interrupted germination except the *Fusarium spp.* Some of the latter were slightly pathogenic to seedlings of crested wheatgrass a week or more after germination but the pathogenic isolates in all cases produced direct necrosis, most often near or above the seed, with resultant stiff, lustrous seedlings rather than dull, wilted ones such as commonly occur in field soil. *Fusarium spp.* therefore were not included in further studies.

Because of the relative infrequency of *Rhizoctonia spp.* in isolation plates of greenhouse and field-grown seedlings, these fungi were not included in soil infestation trials.

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM AND HELMINTHOSPORIUM SATIVUM AT THREE DEPTHS OF INFESTATION

Entire, potato-dextrose-agar, Petri-dish cultures of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* were placed in steamed soil in 4-inch clay pots at three depths as follows: one-eighth inch, 1 inch, and 2 inches below 50 planted crested wheatgrass seeds. Growths of *P. graminicola* and *P. debaryanum* cover a Petri dish of potato-dextrose agar in 48 hours; *H. sativum* requires a week. The cultures used therefore were 2 and 7 days old, so that all were actively growing and about the same size when placed in the steamed soil. The seeds in all cases were covered to a depth of one-half inch with soil. The soil was kept moist, not wet, with tap water.

The experiment was repeated. The first trial included 2 isolates of *Pythium graminicola,* 1 of *P. debaryanum,* 2 of *Helminthosporium sativum,* and 1 check of sterile agar. The second trial included 2 other isolates of *P. graminicola,* the same and 1 other isolate of *P. debaryanum,* the same 2 isolates of *H. sativum,* and a check of sterile agar. Each check and each isolate of the 3 fungi at 3 depths was represented by duplicate pot cultures in each of the trials.

Original stands and numbers of living, dead, and obviously diseased seedlings 1 month after planting were recorded and are presented in table 1. Six weeks after planting, 1 replicate in the first trial and both replicates in the second trial were washed free of soil, the tops and roots separated, air dried, and weighed. These weights are presented in table 2.

There was failure of germination in all pot cultures infested with *Pythium graminicola,* even when infestation was at a depth of 2 inches. Moreover, of the resultant seedlings, only an occasional one was green and vigorous (Fig. 1, A). Those listed as healthy were so listed because there was no evidence above the soil line of direct or indirect necrosis. Furthermore, the profuse wilting and indirect necrosis of seedlings in *P. graminicola*-infested soil was comparable to that in field soil.

Pythium debaryanum failed to induce disease or to kill plants after seed germination was completed. On the other hand, *P. debaryanum* ob-

viously destroyed many germinating seeds when placed one-eighth or 1 inch below the seed, but not when placed 2 inches below the seed (Fig. 1, B). Alfalfa seeds subsequently planted in these pots failed to germinate or damped-off. It is assumed therefore that *P. debaryanum* was present and active during germination of the crested wheatgrass seed.

In general, *Helminthosporium sativum* interrupted germination less than *Pythium debaryanum* and *P. graminicola*, and not at all when placed 1 inch and 2 inches below the seed (Fig. 1, D). In 1 of 4 cases (trial 2,

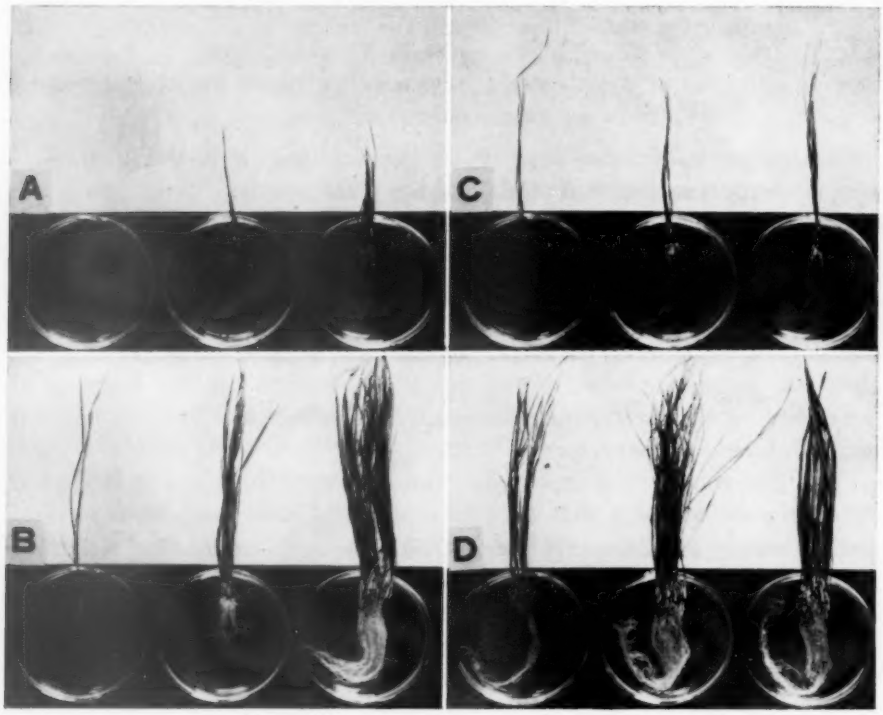

FIG. 1. Crested wheatgrass seedlings grown in steamed soil infested (left to right) one-eighth, one inch, and two inches below the seed with (A) *Pythium graminicola*, (B) *P. debaryanum*, (C) *Helminthosporium sativum*, isolate $12C_2$, and (D) *H. sativum*, isolate $8C_2$.

isolate 12 C_2) interruption of germination was considerable at all depths (Fig. 1, C). *H. sativum* was more pathogenic to seedlings than *P. debaryanum*, much less so than *P. graminicola*, and like *P. debaryanum*, had no effect when placed at some distance from the seed, especially in trial 1. Furthermore, *H. sativum* induced considerable direct necrosis on turgid, lustrous plants, but very little wilting and indirect necrosis. It should be pointed out that the over-all effects of *H. sativum* isolates 8 C_2 and 12 C_2 tended to be reversed in trials 1 and 2. No clue to this inconsistency can be offered.

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM SATIVUM ON PLANTS OF DIFFERENT AGES

The experiments in which steamed soil was infested at different depths suggested the possibility that crested wheatgrass seedlings were susceptible

TABLE 1.—*Healthy, diseased, and dead seedlings of crested wheatgrass in steamed soil infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum at depths of one-eighth, 1 inch, and 2 inches below the seed. (Average of 2 replicates of 50 seeds each)*

Fungus species	Depth of infestation below seed	Number of seedlings in Trial 1				Trial 2			
		Healthy	Diseased	Dead	Total	Healthy	Diseased	Dead	Total
	Inches		*Isolate 1 C*				*Isolate 4 C*		
Pythium graminicola	⅛	1[a]	1	0.5	2.5	0	1	1.5	2.5
	1	4	4	6	14	2.5	4	12	18.5
	2	6.5	8	9	23.5	8.5	5	13.5	27
			Isolate 3 C				*Isolate 8 C*		
	⅛	0	0	0.5	0.5	1	0.5	1.5	3
	1	1	2	6	9	4.5	2.5	12	19
	2	2	15	6	23	8.5	8	12.5	29
			Isolate 6 C				*Isolate 6 C*		
P. debaryanum	⅛	5.5	1	0.5	7	2	0	1	3
	1	0.5	0.5	0	1	13.5	0	0	13.5
	2	31	0	0	31	36	0	0	36
			Isolate 6 C				*Isolate 7 I*		
	⅛	3.5	1	0	4.5	2.5	0	0	2.5
	1	6	0	0	6	34	0	0	34
	2	16.5	0	0	16.5	39	0.5	0	39
			Isolate 8 C_2				*Isolate 8 C_2*		
Helminthosporium sativum	⅛	1.5	1.5	7.5	10.5	15	0.5	5	20.5
	1	13	2	6	21	42	1	0	43
	2	17.5	5	2.5	25[b]	28	4.5	0.5	33
			Isolate 12 C_2				*Isolate 12 C_2*		
	⅛	0.5	0	8	8.5	2.5	4	5.5	12[b]
	1	25.5	1.5	2.5	29.5	2	2	5	9
	2	33.5	1	0	34.5	0	1	1.5	2.5
Check	⅛	28	0	0	28	41.5	0	0	41.5
	1	29	0	0	29	39.5	0	0	39.5
	2	28	0	0	28	40.5	0.5	0	41

[a] In *Pythium graminicola*-infested soil, "healthy" seedlings were living and not obviously diseased, but stunted.
[b] Stunted.

at all ages to *Pythium graminicola* but that only nongerminated or germinating seed was susceptible to *P. debaryanum*. The variable results with *Helminthosporium sativum* indicated no distinct age-susceptibility pattern. Since seedlings growing in field soil exhibit symptoms for some time, it is a sound assumption that the causal agent of seedling blight is pathogenic to seedlings at least 2 weeks old.

Plants of different ages were exposed to *Pythium graminicola*, *P. debaryanum*, and *Helminthosporium sativum* in the following manner: Strips of growing Petri-dish cultures of the 3 fungi were laid close alongside rows of crested wheatgrass 5, 8, 12, 15, 22, and 28 days old. The youngest plants, from seed planted 5 days previous, were in reality seeds

TABLE 2.—*Weights, in grams, of dried tops and roots of crested wheatgrass grown in steamed soil, noninfested, and infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum at depths of one-eighth, 1 inch, and 2 inches below the seed*

Fungus species	Depth of infestation below seed	Weight, in grams, in			
		Trial 1		Trial 2	
		Tops	Roots	Tops	Roots
	Inches	*Isolate 1C*		*Isolate 4C*	
Pythium graminicola	⅛	0	0	0	0
	1	0.04	0.02	0.01	0.01
	2	0.17	0.17	0.03	0.03
		Isolate 3C		*Isolate 8C*	
	⅛	0	0	0.01	0.01
	1	0.01	0.01	0.02	0.02
	2	0.10	0.10	0.04	0.05
		Isolate 6C		*Isolate 6C*	
P. debaryanum	⅛	0.70	0.40	0	0.01
	1	0.06	0.03	0.03	0.03
	2	1.33	1.51	0.36	0.11
		Isolate 6 C		*Isolate 7I*	
	⅛	0.16	0.09	0.04	0.02
	1	0.51	0.45	0.33	0.14
	2	0.94	0.91	0.30	0.12
		Isolate 8C₂		*Isolate 8C₂*	
Helminthosporium sativum	⅛	0.08	0.06	0.01	0.01
	1	0.74	0.47	0.24	0.12
	2	0.20	0.15	0.18	0.09
		Isolate 12C₂		*Isolate 12C₂*	
	⅛	0.17	0.10	0.04	0.02
	1	1.22	1.03	0.04	0.05
	2	1.42	1.15	0.02	0.03
Check	⅛	1.77	1.53	0.30[a]	0.13
	1	1.26	2.06	0.40	0.13
	2	1.45	1.25	0.35	0.12

[a] Plants in trial 2 washed 6 weeks after seed planted; in trial 1, 10 weeks after seed planted.

in late stages of germination. The cultures were laid one-half to three-fourths inch below the soil surface about one-half inch to the outside of 1 row in each pot. The other row, about 2 inches distant, was left undisturbed and was intended as a check. Each row consisted of the seedlings from 25 seeds. The experiment was repeated; the first trial consisted of 4 replicates of 1 isolate of each fungus; the second consisted of 2 replicates of each of 2 isolates of each fungus.

After 3 days, a few of the youngest seedlings appeared wilted by each fungus, the most by *Pythium graminicola*, the fewest by *Helminthosporium sativum*. A few young seedlings may have been mechanically injured during the introduction of the cultures. After 5 days, plants of all ages in rows exposed to *P. graminicola* were wilting, particularly the lower leaves (Fig. 2, A), but there were no additional observable pathogenic effects in the rows exposed to *P. debaryanum* (Fig. 3, A) or *H. sativum* (Fig. 4, A).

After 21 days *Pythium graminicola* had killed all the seedlings in the youngest exposed rows and over half the seedlings in exposed rows 3 and 7 days older. Seedlings in exposed rows of all ages were unmistakably wilted

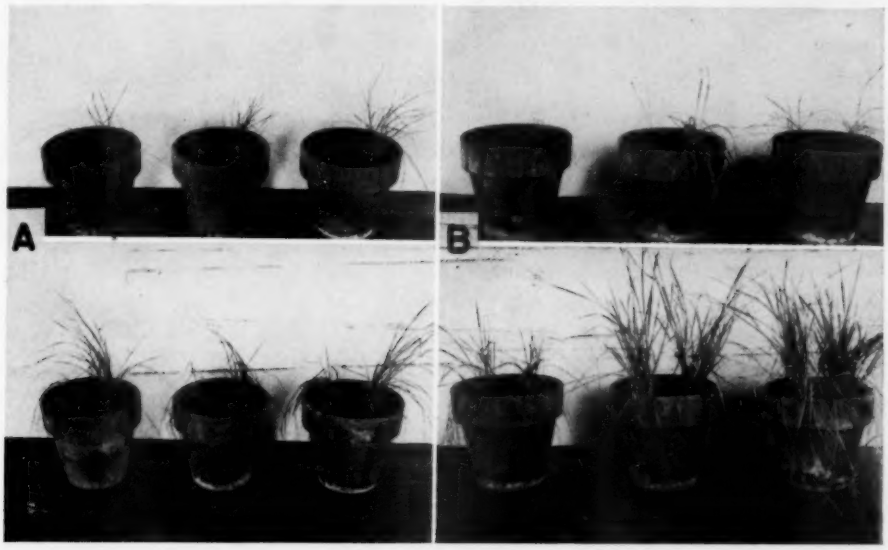

FIG. 2. Crested wheatgrass seedlings exposed to *Pythium graminicola* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

and their lower leaves were undergoing indirect necrosis (Fig. 2, B). Furthermore, all the unexposed rows except perhaps those of the 2 oldest series had wilted and developed symptoms typical of plants infected by *P. graminicola*. Evidently the pathogen had become estabished in the root zone of plants in the rows intended as checks (Fig. 2, B). In the meantime there was no evidence of further development of top symptoms in rows exposed to *P. debaryanum* or *H. sativum* (Fig. 3, B and 4, B).

Ultimately there was partial recovery of some seedlings in all but the youngest rows exposed to *Pythium graminicola*. As a final measure of the effects of these three fungi on seedlings exposed at different ages in this experiment, 1 replicate of the first trial was washed out, air dried, and weighed. The weights of roots and tops were in accord with the appearance

FIG. 3. Crested wheatgrass seedlings exposed to *Pythium debaryanum* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

of the plants; only plants exposed to *P. graminicola* were light in weight, especially those very young when exposed.

It is obvious that *Pythium graminicola* was pathogenic to crested wheatgrass seedlings at least 28 days old, and that *P. debaryanum* and *Helminthosporium sativum* had little or no effect on any but very young seedlings. The results in the second trial were the same as in the first.

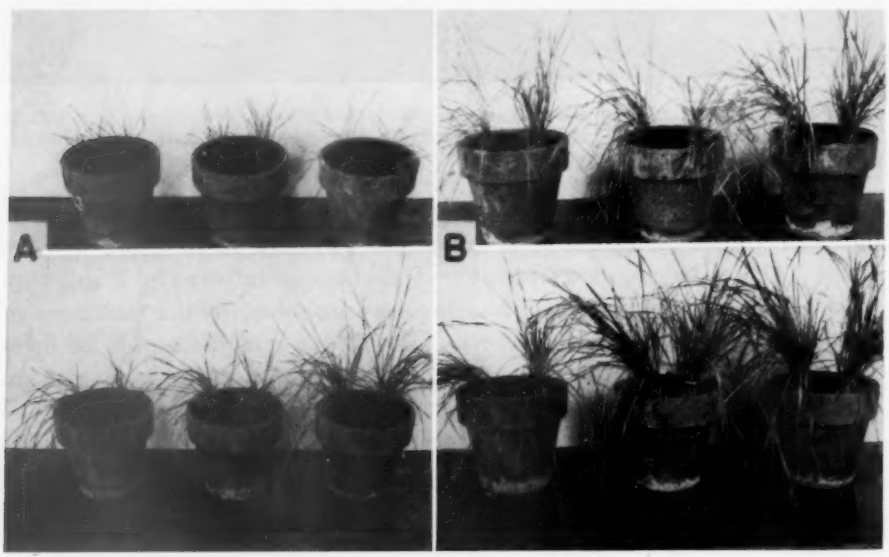

FIG. 4. Crested wheatgrass seedlings exposed to *Helminthosporium sativum* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM SATIVUM ON CRESTED WHEATGRASS SEEDLINGS IN NONSTEAMED BROMEGRASS SOD

Experiments thus far reported were with steamed soil. The shortcoming of assuming pathogenicity of root-infecting fungi on the basis of infestation tests with steamed soil are obvious and have been demonstrated repeatedly. During the course of other investigations, nonsteamed soil from a plot of bromegrass sod had repeatedly yielded stands of healthy crested wheatgrass seedlings comparable to those in steamed soil.

Pots of this bromegrass sod soil, nonsteamed, were infested with entire Petri-dish cultures of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum.* The cultures were placed at a depth of one-half inch

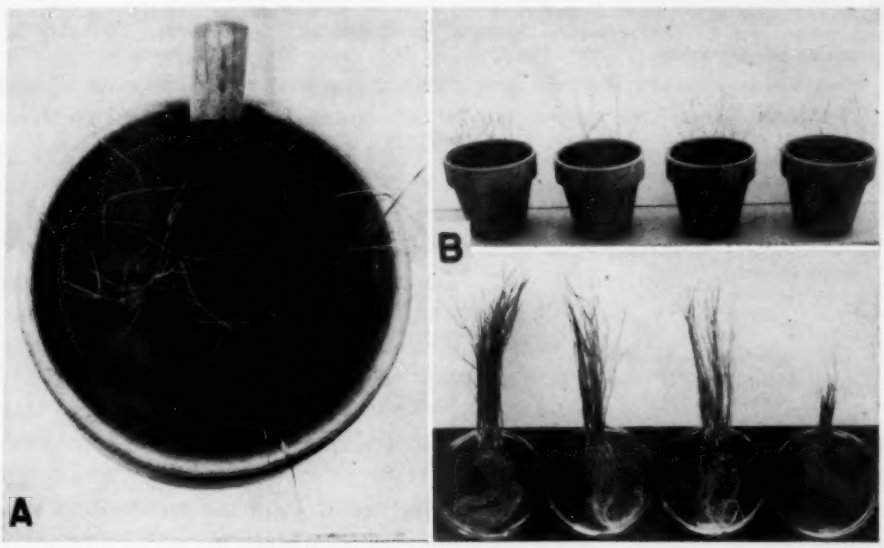

FIG. 5. A. Typical wilting of crested wheatgrass in early stages of seedling blight in infested field soil. B. Crested wheatgrass seedlings grown in non-steamed bromegrass sod. Left to right, sod not infested, infested with *Helminthosporium sativum,* infested with *Pythium debaryanum,* and infested with *P. graminicola.*

below 50 crested wheatgrass seeds planted in each pot. Number of seedlings and air-dry weights of tops and roots are presented in table 3. There was considerable interruption of germination in pot cultures infested with *P. graminicola,* and the seedlings in these pots were wilted and stunted; some were killed (Fig. 5, B). There were no obvious effects from infestations with *P. debaryanum* and *H. sativum.* Results were the same in a second trial. Each trial consisted of 3 replicates of the check and 2 isolates each of *P. graminicola, P. debaryanum,* and *H. sativum.*

It is obvious that in these experiments with nonsteamed field soil, *Pythium graminicola* was pathogenic to germinating seed and seedlings of crested wheatgrass and *P. debaryanum* and *Helminthosporium sativum* were not.

RECOVERY OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM SATIVUM FROM GERMINATING SEED AND YOUNG SEEDLINGS OF CRESTED WHEATGRASS AT VARIOUS STAGES OF GROWTH

Soil infestation trials revealed that *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* can interrupt germination of crested wheatgrass seed, but that only *P. graminicola* is capable of stunting, wilting, and killing seedlings under a variety of circumstances, and particularly in nonsteamed soil. Evidence that *P. graminicola* is the cause of crested wheatgrass seedling blight would seem to be complete, except that the sequence of symptoms is different in field soil than in soil infestation experiments.

TABLE 3.—*Numbers and weights of tops and roots of crested wheatgrass seedlings grown in nonsteamed bromegrass sod infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum. Averages of 3 replications. Weights in grams on air-dry basis*

Fungus species and isolate	No. of seedlings	Weight, in grams, of Tops	Roots
Pythium graminicola			
Isolate 1	3.3	0.45	.18
Do 2	2.3	0.34	.10
P. debaryanum			
Isolate 1	27.3	2.37	1.51
Do 2	31.0	2.68	1.70
Helminthosporium sativum			
Isolate 1	31.0	2.26	1.56
Do 2	31.6	2.17	1.13
Check	28.6	1.95	.98

It will be recalled that a growing culture of *Pythium graminicola* was pathogenic immediately to germinating crested wheatgrass seed or to seedlings of any age up to at least 28 days. On the other hand, the sequence of symptoms in field soil is: (1) more or less failure of germination, (2) apparently normal development of most seedlings for about a week followed by (3) wilting, stunting, and death of the seedlings or indirect necrosis of the lower leaves.

An attempt was made to reconcile this apparent difference in sequence of symptoms in experimentally-infested steamed soil and naturally-infested field soil by isolating at intervals from seeds germinating and seedlings growing in the latter.

Four soil samples were chosen, 1 each from plots continuously cropped to wheat, peas, sorghum, and alfalfa. The first 2 were known to be heavily infested with both *Pythium debaryanum* and *P. graminicola;* the sorghum plot was likewise infested, but especially heavily with *P. debaryanum.* Soil from the alfalfa plot had repeatedly yielded stands of healthy crested

wheatgrass seedlings comparable to those obtained in steamed soil and was assumed to be free of *P. graminicola.*

These soil samples were distributed, each in a series of pots, with 25 seeds planted in each pot. All the germinating seed or seedlings were removed from 2 pot cultures of each soil sample series on the first, fourth, and eighth day after planting. All the seedlings were removed from 1 pot culture of each series on the eleventh, fifteenth, and twenty-second day after planting. A convenient number of seedlings (6 or 12) was removed from 1 pot culture of each series on the thirty-third day after planting. All germinating seeds and seedlings removed were washed and placed on water agar plates and the resulting growths from them of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* were observed and recorded.

The following facts and trends, taken from the results as presented in table 4, are pertinent. Most of the occurrences of *Pythium debaryanum* were in isolations from germinating seed or from seed or seedlings up to 11 days old (15 days after planting). In the soil from the continuous wheat and continuous peas plots, all but 3 occurrences of *P. debaryanum* were in isolations from seedlings up to a week old (11 days after planting). *P. graminicola* appeared only once in isolations from germinating seeds. It developed far more frequently from seedlings 11 days old (15 days after planting) or older. The abundant appearance of *P. graminicola* on isolation plates preceded somewhat, or coincided with, the occurrence of diseased seedlings among those from which the isolations were made. In general, with an increase in age of seedlings there was a tendency for less *P. debaryanum* and more *P. graminicola* to develop in isolations from them. The occurrence of *Helminthosporium sativum* in isolation plates conformed to no particular time pattern in relation to the occurrence of seed rotting or seedling blight.

These relationships are also worthy of note: In other extensive experiments, seedling blight in soil from the plot of continuous peas was as bad as or slightly worse than in soil from the continuous wheat plot. The isolation results indicate the presence of little or no *Helminthosporium sativum* in the soil cropped to peas as compared with the continuous wheat soil. Furthermore, in the same experiments, there was more seed rotting in the continuous sorghum soil than in the continuous wheat soil. Here again, the isolation results indicate less *H. sativum* in the sorghum soil than in the continuous wheat soil. It is evident that considerable seed rotting or considerable seedling blight did occur in field soil with either an abundance or scarcity of *H. sativum* as indicated by isolation results.

In general then, it appears from these isolation results that *Pythium debaryanum* rather than *P. graminicola* is associated with germinating seed of crested wheatgrass in field soil. Conversely, *P. graminicola* rather than *P. debaryanum* is associated with the roots of seedlings with blight symptoms, particularly at the time those symptoms are occurring.

All of these associations are in accordance with the observed potentialities of *Pythium debaryanum* and *P. graminicola* in pure culture, soil infestation experiments, excepting one. The lack of *P. graminicola* in isolations from germinating seed, even in soil in which it later parasitizes the roots of seedlings profusely, can not be accounted for on the basis of the evidence presented. That lack, however, seemingly constitutes a reasonable basis for the normal growth of seedlings for at least a week in heavily infested field soil.

TABLE 4.—*Occurrences of Pythium graminicola, P. debaryanum, and Helminthosporium sativum in isolations at intervals from crested wheatgrass seedlings growing in four non-steamed soils*

Soil source	Days after planting	Number of isolations	Occurrences of *Pythium graminicola*	Occurrences of *Pythium debaryanum*	Occurrences of *Helminthosporium sativum*	Disease
Continuous wheat	1	43	1	17	1	Present[a]
	4	45	0	6	17	do[a]
	8	48	5	11	5	Present
	11	22	7	8	2	do
	15	19	10	1	1	do
	22	17	6	0	1	Abundant[b]
	33	12	8	1	0	do[b]
Continuous peas	1	38	0	19	0	Present[a]
	4	47	0	23	1	do[a]
	8	44	9	8	3	Present
	11	21	4	7	2	do
	15	18	17	0	1	Abundant
	22	14	2	1	1	Abundant[b]
	33	12	8	0	1	do[b]
Continuous sorghum	1	43	0	27	1	Present[a]
	4	45	0	27	0	do[a]
	8	45	0	20	0	Absent
	11	22	0	12	0	do
	15	21	0	13	0	do
	22	20	11	0	3	Present[b]
	33	12	2	0	0	do[b]
Continuous alfalfa	1	42	0	7	0	Present[a]
	4	31	0	1	1	do[a]
	8	41	1	0	1	Absent
	11	22	0	7	0	do
	15	22	0	0	0	do
	22	21	0	0	0	do
	33	6	0	0	0	do

[a] Germinating seed diseased.
[b] Seedling blight.

SUMMARY AND CONCLUSIONS

Crested wheatgrass seed, when planted in field soil in which seedling stands fail: 1) may fail to germinate as a result of rotting in early stages of germination; 2) may germinate and give rise to seedlings which develop normally for at least a week, sometimes 2 or 3 weeks; after which occurs 3) wilting, stunting, and indirect necrosis of leaves or entire plants.

An attempt to reproduce this symptom sequence involved these procedures and results:

Isolations from greenhouse-grown seedlings revealed the presence of *Pythium graminicola, Fusarium spp., P. debaryanum,* and *Helminthosporium sativum* in that order of predominance. *Rhizoctonia spp.* were recovered occasionally. All these fungi except *Rhizoctonia* were taken from field-grown crested wheatgrass seedlings.

In infestation experiments with steamed soil, *Pythium graminicola* was pathogenic to germinating seed and seedlings of crested wheatgrass even when placed 2 inches from the seed and was pathogenic to seedlings up to 28 days old. *P. graminicola* induced wilting comparable to that of seedlings in nonsteamed field soil. *P. debaryanum* was pathogenic to germinating seed when placed one-eighth or 1 inch below the seed, but not when placed 2 inches below the seed. *P. debaryanum* possibly was pathogenic to very young seedlings. *P. debaryanum* did not induce typical wilting. *Helminthosporium sativum* varied in its effects, but in general was pathogenic to germinating seed when placed near it and was noticeably pathogenic only to very young seedlings. *H. sativum* did not induce typical wilting.

In nonsteamed soil taken from a plot of bromegrass sod, only *Pythium graminicola* induced typical wilting and stunting of crested wheatgrass seedlings.

In isolations at intervals from germinating seed and seedlings of crested wheatgrass in lots of soil from plots variously infested, there was a tendency for less *Pythium debaryanum* and more *P. graminicola* to appear on isolation plates with increase in time after planting. Abundant occurrence of *P. graminicola* preceded somewhat or coincided with the occurrence of diseased seedlings among those from which isolations were made. The occurrence of *Helminthosporium sativum* in isolation plates conformed to no particular time pattern in relation to the occurrence of seed rotting or seedling blight. There was no definite relationship between incidence of seed rotting or seedling blight and the frequency of occurrence of *H. sativum* on isolation plates.

In these experiments, *Pythium graminicola* clearly duplicated the symptoms of seedling blight and may be considered its causal agent. The absence of *P. graminicola* on germinating seed, plus the abundance there of *P. debaryanum,* with its demonstrated capacity for interrupting germination, leads to the conclusion that *P. debaryanum* is the principal cause of seed rotting. *Helminthosporium sativum* apparently may be a factor in both seed rotting and blighting of seedlings, but a minor, atypical, and inconsistent one.

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TRANSMISSION OF SUGAR BEET YELLOW-NET VIRUS BY THE GREEN PEACH APHID[1]

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The yellow-net virus disease of sugar beets has been described previously by the author (14). Briefly the symptoms consist of a bright yellow chlorosis of the veins and veinlets of affected leaves. The vector, the green peach aphid, *Myzus persicae* (Sulzer), has been reported, but details of the relationships between the vector and the virus have not been fully described. This paper presents data relevant to that phase of the work.

MATERIALS AND METHODS

The virus source was originally obtained at Sherman Island, California, and plants inoculated from these original diseased beets were used as virus sources.

The noninfective aphid colonies were maintained upon healthy sugar beet plants in the greenhouse. The colonies were routinely checked for noninfectivity. The aphid transfers were made by the standard camel's-hair brush technique.

The test plants were grown from seed, planted in flats and transplanted to 4-inch pots. The plants and insects were maintained in the greenhouse, and during inoculation they were kept under caged conditions.

DEFINITION OF TERMS

The following terms are used in the paper in describing the relationships between the vector and the virus:

Infection feeding (18) :—The time that a previously noninfective vector is fed on a virus source.

Test feeding (18) :—The time that a vector is fed upon a healthy recipient host, or test plant.

Pre-penetration time:—A variable period of time elapsing between the time an insect is placed upon a plant and the time that penetration begins. This corresponds to the "penetration time" of Watson (16).

Acquisition threshold:—The minimum time necessary for a vector to feed upon a disease source in order to obtain an infective charge of virus.

Inoculation threshold:—The minimum time necessary for an infective vector to feed upon a healthy plant in order to successfully inoculate that plant with a given virus. The term is equivalent to the "threshold" period as used by Storey (12).

[1] A condensed portion of a thesis presented to the graduate division of the University of California, February 1947, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Latent period:—The term in common usage which denotes the time that must elapse before a vector can be demonstrated to be infective, after surpassing the acquisition threshold. Two common synonyms for this term are "waiting" and "incubation" period (11).

Retention period:—The period of time, following an infection feeding, during which a vector is capable of producing infections if fed upon a succession of susceptible healthy plants.

Infective:—The term commonly used to describe the condition of a vector which has virus present in its body and is potentially capable of transmitting that virus.

Actively infective:—A condition of an infective vector during which it will successfully inoculate a healthy susceptible host, if given a suitable test feeding upon it.

Passively infective:—A condition of infectivity when a vector will not inoculate a healthy susceptible host, even though given an adequate test feeding period upon that host.

PRE-PENETRATION TIME

Early in the work it was recognized that an aphid, when transferred from one host to another, required a certain period of time to recover from the manual act of transference and begin to feed upon the new host plant.

Watson (16) used the term "penetration time" in referring to this phenomenon and reported an average of 4.88 minutes for the completion of the period.

Since aphids, during this period, are not penetrating any tissue in a permanent sense, it is felt that the term "pre-penetration" time might be used with more accuracy to denote the presence of this period. An aphid, during this time, may make trial penetrations, but these are usually but a few seconds in duration. For convenience, in this particular work, these are not considered true penetrations, which usually are relatively permanent and terminate in the phloem tissues of the plant (10, 15).

The importance of the pre-penetration period is realized when attempting to determine the inoculation or acquisition threshold.

Based upon data taken upon 64 watched individuals, the minimum pre-penetration period noted was 30 seconds, while the maximum was 13 minutes, 9 seconds. Individuals which wandered above this maximum were discarded as being abnormal, either inherently, or due to injury during transference. The average pre-penetration time was 4.9 minutes.

ACQUISITION THRESHOLD

The acquisition threshold, as used in this paper, is applied to the shortest time determined that a previously noninfective aphid must feed upon a disease source, before it is able to acquire an infective charge of the virus. The pre-penetration time was allowed to elapse before the timing of the infection feeding period was begun.

To determine this threshold, a single previously noninfective mature aptera of the green peach aphid was placed upon a virus source plant and allowed to complete the pre-penetration period. It then was given an infection feeding period of definite length according to the experiment. The intervals used were 2, 4, 5, 10, 15, 20, and 30 minutes infection feeding upon the diseased source. The results of the trials are shown in table 1.

The results indicate that apterae are capable of acquiring sufficient virus to become infective within 5 minutes after they have placed the rostrum in position for penetration.

Penetrated tissue studies by Roberts (6) indicated that only 11 per cent of the stylet tracts of green peach apterae will attain the phloem of sugar beet leaves in 15 minutes.

Since an acquisition threshold was obtained in a time too short to allow the aphids to reach the phloem tissues, it would appear that the virus is present in tissues of the mesophyll. These results obtained are somewhat comparable to those of Watson (17) in experimental transmission of the beet-yellows virus with the green peach aphid.

TABLE 1.—*Results[a] of trials to determine the acquisition threshold of the yellow-net virus, using single previously noninfective green peach aphids, a variable infection feeding period, and a 24-hour test feeding period*

Infection feeding in minutes							
2	4	5	10	15	20	30	Total
0/5	0/5	4/40	1/75	2/60	0/5	1/20	8/210

[a] In the ratios listed, the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.

INOCULATION THRESHOLD

The inoculation threshold, as defined, is the minimum time necessary for an infective vector to feed upon a healthy plant in order to successfully inoculate that plant with a given virus.

The experimental procedure used to determine this threshold was to allow a single infective aptera of the green peach aphid to feed, following the completion of the pre-penetration period, upon a healthy beet seedling for a given time. The test feeding period was varied according to the specific experiment. In the preliminary work the aphids, after feeding for the specified time on the healthy beet, were transferred to a second healthy beet and allowed to feed for 24 hours. This served as a partial check upon the vector infectivity. The results are given in table 2.

From the data in the table it would appear as if the inoculation threshold is somewhere near 15 minutes.

Watson (17) found, upon 2 occasions, using a total of 25 plants, that the green peach aphid was able to inoculate a plant with the beet-yellows virus in a test feeding period of 7 minutes. This could not be done with the yellow-net virus.

TABLE 2.—*Results[a] of trials to determine the inoculation threshold of the yellow-net virus, using single infective green peach apterae and a variable test feeding period*

	Test feeding in minutes							
	2	5	10	15	20	25	30	Total
Test plant	0/5	0/15	0/15	2/30	1/30	1/30	3/10	7/135
Check plant[b]	.....	5/15	6/15	2/10	.....	.....	.....	13/40

[a] In the ratios listed the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.

[b] The check plant refers to the second beet inoculated by the aphid after completion of the test feeding period upon the first recipient host. The feeding period on the check plant was at least 24 hours.

The results indicate that in order for an infective insect to inoculate a healthy beet seedling, the feeding period must be at least 15 minutes or approximately the period of time required by the insect to reach the phloem region of a beet seedling. Apparently the insect must place the virus in, or near, the phloem region if inoculation is to be achieved.

Determination of the inoculation threshold aids in the interpretation of latent period data, and possibly in ascertaining which tissue region of a plant must be attained by a vector if successful inoculation with a virus is to occur.

LATENT PERIOD

The time necessary for a vector to become infective after having access to a virus has been variously termed as "waiting" or "incubation" period as well as the "latent" period (7, 11). The writer prefers the term "latent period" and would use "incubation period" for designating the time necessary for symptoms of disease to develop within a plant.

The method employed to determine the latent period was as follows. Previously noninfective insects were placed upon a virus source for a given length of time. The length of the infection feeding period was varied from 15 minutes to an hour. At the end of this feeding period the insects were transferred singly to a healthy recipient host and allowed to feed for a given test feeding period. This period was varied from 15 minutes to 45 minutes. At the termination of the test feeding period the vector was removed and the plant fumigated. The results of the trials are given in table 3.

TABLE 3.—*Results[a] of trials to determine the latent period of the yellow-net virus within the green peach aphid, using previously noninfective single apterae, a variable infection feeding period, and a variable test feeding period*

	Time in minutes						
Infection feeding / Test feeding	15 / 15	30 / 15	15 / 30	30 / 30	30 / 45	60 / 30	Total
	0/5	0/5	1/5	1/5	0/5	1/5	3/30

[a] In the ratios listed the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.

The shortest total time period which yielded positive results was 45 minutes. Since the acquisition threshold has been determined to be approximately 5 minutes, and the inoculation threshold approximately 15 minutes, in the total time example of 45 minutes there remained a possible latent period of 25 minutes. This 25-minute interval could be a true latent period, or only an apparent one, depending upon whether it is assumed that one, or the other, or both, of these thresholds was prolonged in the case of the particular trial.

The results are somewhat similar to those obtained by Watson (17), using the green peach aphid as the vector for the sugar beet-yellows virus, which led her to conclude that there was no sharply defined latent period. The same conclusion appears to be justified in the case of the yellow-net virus.

VECTOR EFFICIENCY

Vector efficiency can be defined as the transmission expectancy, expressed as percentage, when a vector is given one access period to a diseased source and subsequently transferred to the first healthy recipient host.

During retention studies it was learned that a given insect often would fail to transmit the virus, as evidenced by the appearance of symptoms, to the first of a series of healthy recipient beets. This will directly affect the vector efficiency results. Therefore, it is felt that the use of the term efficiency, for convenience and for purposes of this paper, should be limited to the results of one transfer of an insect from a virus source to a healthy recipient host. The time spent in the infection feeding and test feeding periods should be long enough to allow a near maximum of infectivity and transmission to occur. At best the vector efficiency rating, under the restrictions of the definition, will give only a partial estimate of the number of insects actually infective.

The problem of vector efficiency was approached by combining data from numerous experiments and the efficiency of a "vector-group" composed of 10 apterae, was compared with the efficiency of single apterae. In the examples used, the infection feeding period was 24 hours, and the test feeding period was 24 hours. Based upon 207 examples, it was found that a vector-group can be expected to transmit the yellow-net virus approximately 75 per cent of the time under the limitations of the definition. The vector efficiency rating for a single aptera, based upon 125 examples, is approximately 26 per cent.

VIRUS RETENTION

Early in the experimental work it was learned that the virus was of the "persistent" type, as defined by Watson and Roberts (19), *i.e.*, the vector, after once becoming infective retains the ability to transmit the virus for a relatively long period of time.

This does not imply that an infective insect will infect every plant that it is fed upon, but rather that when an insect is transferred to a series of healthy plants until its death, some positive plants will appear in the series.

TABLE 4.—*Results[a] of trials to determine the ability of vector-groups, composed of 10 infective green peach aphid apterae, to retain the yellow-net virus. The aphid groups were transferred daily to successive healthy beet seedlings*

Aphid group No.	Day transferred										
	1	2	3	4	5	6	7	8	9	10	11
1	+	+	+	+	+	+	+	+	+	+	+×
2	+	+	+	+	+	+	+	+	+	−	−×
3	+	+	+	+	+	+	+	+	−	−×	
4	+	+	+	+	+	+	+	+	+×		
5	+	+	+	+	+	+	−	+×			
6	0	+	+	+	+	+	−	+	−	−×	
7	+	+	+	+	+	+	+	+	−×		
8	+	+	+	+	+	+	−×				
9	+	+	+	+	+	+	+	+	−×		
10	−	+	0	+	+	−	+×				

[a] The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. The zero sign (0) indicates that the plant died, while the (×) sign indicates the day that all insects were dead, and transfers were discontinued.

Tables 4 and 5 give in detail the results of retention experiments, using both vector-groups and single insects. Table 5 indicates the irregularity of the intervals with which the infection-producing feedings occur when manipulating single insects, whereas in table 4, this irregularity appears in the vector-group only when it has been reduced by mortality to approximately a single individual.

EFFECT OF MULTIPLE FEEDING PUNCTURES UPON VIRUS TRANSMISSION

The appearance of negative plants in series of daily transfers of infective insects to healthy plants has been noted many times. In fact, Freitag (4)

TABLE 5.—*Results[a] of trials to determine the ability of single infective green peach aphid apterae to retain the yellow-net virus. The single apterae were transferred daily to successive healthy beet seedlings. Sixteen of the 30 aphids tested failed to transmit the virus in any of the transfers and are not included in the table*

Single aphid No.	Day transferred						
	1	2	3	4	5	6	7
1	+	−	−	−	−×		
3	+	−	−	−×			
4	−	−	+	+	−×		
7	−	−	−	+×			
9	−	−	−	+×			
10	−	−	−	−	−	+	−×
18	−	−	+	−	−	−×	
21	+	+	−×				
22	+	−	+	+	+	+×	
24	+	+	+×				
25	+	+	−×				
28	−	+×					
29	+	+	−	+	+×		
30	−	−	−	+×			

[a] The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. The (×) sign indicates the day that the insect was dead and transfers were discontinued.

and later Bennett and Wallace (1) used this phenomenon in conjunction with other evidence, as indicating the lack of multiplication of the curly-top virus within its vector, *Eutettix tenellus* (Baker). The explanation of the occurrence of the negative plants will vary according to the initial assumption that is made as to the mechanics of virus transmission by insects.

Two assumptions are possible. One, accepted by Storey (13), is that an infective insect has periods when it is "actively infective", *i.e.*, periods during which it will eject virus during feeding, alternating with periods during which it is "passively infective", *i.e.*, periods during which it does not eject virus, even though it is feeding. Also, the passively infective periods increase in number and duration as the length of time since the last infection feeding increases. This implies that virus ejection by an infective insect is irregular during the course of its feeding, and that the explanation for the "skips" in transmission is that the insect was passively infective, *i.e.*, failed to inject virus, during a particular test feeding.

The second assumption as to the method of virus ejection is that the virus, as long as it is present in the insect, is being ejected continuously as the insect feeds. In this case the reasons for the "skips" in a retention series would be (1) plant resistance, (2) ejection of virus into tissue unfavorable for multiplication or translocation, (3) the injection of a subminimal infective dose of the virus, or (4) any combination of the above mentioned factors.

The influence of the factor of plant resistance has been shown to be operating in certain cases by Giddings (5), and thus in some cases it can be the primary factor operating against transmission. But even in the case of a very resistant host, if any percentage of transmission was established, a typical pattern exhibited in a retention series undoubtedly could be identified which would not be explicable upon the basis of plant resistance.

The importance of the factor of a favorable tissue region for successful virus invasion might be difficult to demonstrate experimentally. Sheffield (9) has shown that individual cells of the same tissue appear to vary in their susceptibility to a given virus. Consequently the existence of such a factor must be borne in mind.

The third factor, that of injection of a subminimal infective dose of virus, is the one upon which the idea of mass inoculation, as interpreted by Carsner and Lackey (2) and Severin (8), depends for its experimental proof. The factor possibly acts in certain cases, but the consistent failure to obtain conclusive experimental data appears to relegate it to a position of minor importance in the consideration of reasons for the appearance of negative plants in a retention series.

In order to test the hypothesis of continuous virus injection during feeding, the following experiment was done, with two variations. A colony of green peach aphids was established on a virus source plant. When the colony was of sufficient size, aphids were removed as pairs, *i.e.*, two aphids

which were feeding on the same vein, or veinlet, or which were feeding as closely together as possible, were selected and each was put on a separate healthy beet seedling. One of the aphids of the "pair" was allowed to feed continuously for the entire test feeding period. The other member of the pair was disturbed at 30-minute intervals so as to force it to make at least 10 punctures.

In one experiment the total test feeding period was approximately 24 hours, and the disturbed aphid of the pair, following the 4-hour discontinuous feeding was allowed to remain feeding upon the same plant until the termination of the test feeding period, or approximately 20 hours. In this experiment a total of 20 aphid pairs was used. The continuously fed aphids successfully inoculated 6 out of 20 plants fed upon, while the discontinuously fed aphids successfully inoculated 8 out of 20 plants fed upon.

In the second experiment, each pair was allowed a total test feeding period of 7 hours, and the disturbed member was forced to move at 30-minute intervals for the first one and one-half hours, at one and one-half hour intervals for the next 3 hours, and at 30-minute intervals for the next two and one-half hours. At the end of the final half hour interval all the plants in the experiment were fumigated. In this experiment a total of 50 aphid pairs was used. The results showed that the continuously fed aphids successfully inoculated 17 out of the 50 plants used, while the discontinuously fed aphids successfully inoculated 13 out of the 50 plants used.

The object of the trials was to determine if the amount of saliva that the insect injects into the plant while making the puncture would affect the amount of effective virus ejected. Also an attempt was made to learn if the factor of tissue region penetrated was operating to any degree of effectiveness against transmission.

Work by Davidson (3) has indicated that usually an aphid, when undisturbed for a period of time, will make but one penetration. It was believed, therefore, that by the above experimental procedure a comparison could be made between the effect of a single puncture and its accompanying salivary secretion and tissue region penetrated, and the effect of 10 punctures and their accompanying salivary secretions and 10 different tissue locations. This comparison was to be made upon similar beet seedlings with two vectors which had fed upon the same virus source, and as nearly as possible, in the same general tissue region on that disease source.

It was believed that the inoculation threshold was one of several influencing factors in the experiments not adequately accounted for, and that it might have influenced the results. Since this threshold was determined to be approximately 15 minutes, it was believed that the disturbing intervals might have been of insufficient length to allow proper completion of the inoculation thresholds in all instances. As a result, another experiment was designed in which the interval between the disturbances was lengthened to 1 hour, to allow an adequate inoculation threshold. In addition, one other variant was introduced, in which a third series of aphids was transferred to a new plant every hour.

All the aphids for the experiment were taken from a colony which had been derived from a single green peach aphid aptera. The aphids were fed upon a single diseased source plant, and then transferred to healthy beets in groups of three, *i.e.*, three aphids feeding upon a common vein, or in close proximity to each other were selected and then placed singly upon three healthy beet seedlings. One of the aphids was left undisturbed for 10 hours (continuous feeding). The second aphid was forced to move, by

TABLE 6.—*Results*[a] *of trials to determine the effect of multiple feeding punctures upon the efficiency of transmission of the yellow-net virus by infective green peach aphid groups, composed of three aphids, fed upon the same virus source. One aphid of each group was allowed to feed continuously for 10 hours, one was disturbed in its feeding at the end of each hour of feeding, until 10 punctures had been made, while the third aphid was transferred to a new beet seedling at the end of each hour, until it had fed upon 10 plants*

Aphid group No.	Continuous feeding	Discontinuous feeding	Hourly serial transfer plant No.										
			1	2	3	4	5	6	7	8	9	10	Total[b]
1	+	+	−	−	−	−	−	−	−	−	−	−	0
2	+	+	+	+	−	−	−	−	−	+	−	−	3
3	+	+	−	−	−	−	−	+	−	+	+	+	4
4	+	−	−	−	−	−	−	−	0	−	−	−	0
5	+	+	−	−	−	−	−	−	−	+	−	−	1
6	+	−	−	−	−	−	−	−	+	−	−	−	1
7	−	−	−	−	−	−	−	+	−	−	+	+	3
8	−	−	−	+	−	−	−	+	−	−	−	−	2
9	−	+	−	−	−	−	+	−	−	+	−	−	2
10	−	−	−	−	−	−	−	+	−	−	−	−	1
11	−	+	−	−	−	−	−	−	−	−	+	−	1
12	+	+	−	−	−	−	−	−	−	−	−	−	0
13	−	+	+	−	−	+	+	−	−	−	−	+	4
14	−	−	−	+	−	−	−	−	−	−	−	−	1
15	+	+	−	−	−	−	+	−	−	−	−	−	1
16	−	−	+	+	−	−	−	−	−	−	−	−	2
17	−	+	−	−	−	+	+	−	+	+	+	−	5
18	+	+	+	−	−	−	−	−	−	−	−	−	1
19	−	+	−	−	−	−	+	−	−	−	+	−	2
20	−	+	0	−	−	−	−	+	−	−	−	−	1
21	−	+	−	+	+	−	−	+	−	−	−	−	3
22	−	+	0	−	−	−	−	−	−	−	−	−	0
23	−	−	+	−	+	−	−	+	−	−	−	−	3
24	−	+	+	+	+	−	−	−	−	+	+	−	5
25	−	+	−	+	−	−	−	−	−	−	−	−	1
Total	9	17	6	7	3	2	5	7	2	6	6	3	47

[a] The plus sign (+) indicates the production of the disease and the minus sign (−) shows that no disease resulted. The zero sign (0) indicates that the plant died.

[b] This total represents the total number of plants infected by each aphid in the serial transfers, out of a possible 10 fed upon.

probing with a camel's hair brush, at the end of each hour until it had made 10 moves, and theoretically 10 punctures (discontinuous feeding). The third aphid of the group was transferred to a new beet seedling at the end of each hour until it had fed upon 10 plants (serial feeding). This procedure was repeated 25 times. The seedling beets used in the experiment were either in the cotyledon stage or had one true leaf emerging. The plants were fumigated with nicotine at the termination of the experiment,

and were placed upon one bench in the greenhouse for incubation. The data obtained are in table 6. The results indicate that increasing the number of punctures made by a single infective insect upon a single plant has a beneficial effect upon the transmission of the yellow-net virus. This increase in vector efficiency has statistical support ($\chi^2 = 5.12$ for 1 degree of freedom). It also is evident that the effective salivary ejection, as far as the virus transmission is concerned, must be initial, since the total time spent by the two groups in the test feeding period was the same, *i.e.*, virus transfer occurs during penetration or shortly after the puncture is terminated. Actually the effective feeding time was much shorter in the discontinuous feeding trials, because of the influence of the inoculation threshold, and yet the increase in infection percentage was found in these trials.

The actual increase in the number of infections obtained in the discontinuous trials could have been due to (1) overcoming a plant resistance factor, by the introduction of more virus-bearing saliva as a result of multiple punctures, by attaining a favorable tissue region, etc., or it could have been due to (2) discontinuous effective virus contamination of the salivary secretion, *i.e.*, the insects had both passively and actively infective phases during the test feeding periods, and the procedure accentuated the effect of the actively infective phases.

In the second phase of the experiment an attempt was made (1) to determine the efficiency of the insects by again forcing them to feed 10 times, and also (2) to reduce the effectiveness of a possible factor of plant resistance by allowing the aphids a choice of 10 plants. If the effective salivary ejection occurred during the initial stage of penetration, the demonstrable percentage of infective insects would have been as it was in the discontinuous series. This was true, with a slight, but not significant ($\chi^2 = 1.74$ for 1 degree of freedom), increase in the serial trials.

This increase might have been due to the operation of a plant resistance factor, but if this was the case, its significance was slight. In the serial transfer trials, negative series of plants occurred in 4 cases, while 8 occurred in the discontinuous trials, and it would seem improbable that this would have been due to plant resistance. However, it readily could be explained by assuming the insect was actually noninfective or was in a long passively infective state.

The same reasoning can be applied to the nine examples in which only one infection out of a possible 10 occurred in the serial transfers. Here again it would appear as if the series of skips was due to the insects being passively infective for a series of plants, then actively infective, and again passively infective. This concept of the manner in which the aphids transmit the yellow-net virus would appear to be the more probable explanation of the results obtained in the experiment.

These data, along with those obtained in retention trials, seem to support the opinion that the actual injection of virus into the plant via the salivary secretion is irregular, and that as the infective insect grows older, the periods

of time during which the insect is passively infective are lengthened considerably (4).

TRANSMISSION BY MORPHOLOGICAL VARIANTS

It was decided to determine whether or not the various morphological forms of the aphid species would transmit the virus, and whether or not the power of an individual to transmit the virus would be retained through a moulting period. A colony of green peach aphids was established on a diseased source plant and allowed to increase until "pupae" (young nymphs possessing wing pads which indicate that they will develop into winged, or alate, forms) were present along with the apterae. Pupae from the disease source were placed singly upon healthy beet seedlings, and

TABLE 7.—*Results[a] of trials to determine whether or not the yellow-net virus could be retained by an individual aphid through a moulting period. Single infective green peach aphid pupae were transferred daily to healthy beet seedlings until they died. If a nymph moulted during the course of transference, the resulting alate was transferred daily until its death. The apterae, fed the first day only, served as a check upon the virus source*

Pupa No.	Day transferred									Aptera No.	Day transferred
	1	2	3	4	5	6	7	8	9		1
1	–	–	+	–	–/	–	–×			1	+
2	–	+	+×							2	–
3	+	–	–×							3	–
4	–	–/	–×							4	+
5	–	–	–×							5	–
6	–/	–	–	–	–	–	–×			6	+
7	–/	–	+	–	–×					7	–
8	–	+	–	–/	–×					8	–
9	–/	–	–	–	–	–	+×			9	–
10	–	–	–	–	–	+/	–	–	+×	10	–
11	–×									11	–

[a] The plus sign (+) indicates the production of the disease, and the minus sign (–) shows that no disease resulted. The diagonal line (/) indicates the day on which the pupa moulted producing the alate, and the (×) sign shows the day on which the insect was found dead.

were subsequently transferred daily until death to healthy seedlings. A comparable number of apterae from the same colony were set up singly on the first day to serve as a check upon the virus source. During the course of the serial transfers some of the pupae moulted and produced the alate form. These resulting alatae were transferred daily until death of the individual occurred. The results of the trials are given in table 7.

Five of the pupae and 2 of the resulting alatae transmitted the virus, and in one case the virus was transmitted by a pupa and the alate resulting from that pupa. This would indicate that the virus can be retained by the vector through a moulting period, and that the two main morphological variations of the viviparous form are capable of transmitting the yellow-net virus.

Retention studies have shown that mature apterae will transmit the virus until the time of their death. An experiment was made to determine the acquisition and transmission of the virus by young nymphs. A mature aptera placed on a virus source plant for 24 hours was transferred together with one of its young to individual healthy beet seedlings. Twice in 16 replications was the virus transmitted by the first instar nymphs, thus completing the knowledge of the range of infectivity to include all common stages of the insect.

TRIALS WITH OTHER SPECIES AS VECTORS

The following species of aphids were tested to determine whether or not they would act as vectors of the yellow-net virus: *Aphis rumicis* Linnaeus, *Macrosiphum solanifolii* (Ashmead) (= *gei* (Koch)), *Myzus circumflexus* (Buckton) and *M. pseudosolani* Theobald (= *convolvuli* (Kalt.), = *solani* (Kalt.) of authors). The results of the trials are given in table 8.

TABLE 8.—*Results of trials to determine whether or not several species of aphids will act as vectors of the yellow-net virus. A minimum of 4 and a maximum of 10 individuals were used in each test*

Aphid species	Number of plants inoculated	Number of plants infected
Aphis rumicis Linnaeus	42	1
Macrosiphum solanifolii (Ashm.)	35	3
Myzus circumflexus (Buckton)	25	0
M. pseudosolani Theobald	28	1

A minimum of 4 and a maximum of 10 insects was used in each trial, and all forms, apterae, nymphs, and alatae, were included in the tests. The results indicate that three other species of aphids, viz., *Aphis rumicis* Linn., *Macrosiphum solanifolii* (*Ashm.*) and *Myzus pseudosolani* Theob., aside from the green peach aphid, can serve as vectors of the yellow-net virus of sugar beets under experimental conditions.

In the field the green peach aphid far surpasses any other aphid species in prevalence on sugar beets. *Aphis rumicis* Linn. can be collected in the field on sugar beets, but infestations of this species are localized and irregular.

DISCUSSION

The experimental results which have been obtained during the work have indicated the operation of some of the factors which influence the transmission of the yellow-net virus of sugar beets by the green peach aphid.

The yellow-net virus has been classified as a persistent aphid-borne virus. The terms persistent and non-persistent were introduced by Watson and Roberts (19). Persistent indicates that the virus is retained within the vector for a relatively long time, while non-persistent indicates a relatively short period of retention. Later Watson (18) expressed doubt as to the

value of the degree of retention as the best criterion of separation of the two types of viruses, believing that the influence of a pre-infection feeding fast upon resultant vector efficiency was a more valid measure of distinction. The fast increases the vector efficiency in the case of the non-persistent virus, but does not in the case of the persistent virus. If this latter phenomenon alone is used for separation, then the use of the two terms has lost the word association value, a feature important to the definition of the terms.

One other point needs consideration when using the influence of a pre-infection feeding starvation period upon the vector efficiency as a criterion for persistence or non-persistence. In the case of the non-persistent aphid-borne viruses, the starvation effect is pronounced and can be readily demonstrated. In the case of the persistent aphid-borne viruses, it is doubtful that the factor can be measured under comparable conditions, because of the influence of the relatively long acquisition (5 min.) and inoculation (15 min.) thresholds, which would tend to mask, if not eliminate, this ephemeral phenomenon. The factor has possibilities of being tested in the case of some of the leafhopper transmitted viruses, but again this group of viruses may be unrelated to the aphid-borne persistent viruses.

If it can be considered that the period of retention is relative, and other factors are constant, then there appears to be but little difficulty in classifying a virus either as persistent or non-persistent. It makes little difference whether a virus is retained for 48 hr., 72 hr., or for the functional life of the insect, as long as there exists a number of viruses which will fit into the category of persistent aphid-borne viruses. Likewise it matters little if a virus is retained 15 minutes, or for 6 hours, it still could be considered as a non-persistent virus, relatively speaking, and as long as there is a number of examples of viruses which can be placed into the category, the term serves its purpose. There will be viruses which will be in the transition zone, as to persistence in the vector, and therefore upon this factor alone they could not be classed either as persistent or non-persistent. However, there appear to be 3 factors which would aid in determining the classification of a virus: (1) the period of retention, (2) the effect of a pre-fasting period prior to the infection feeding upon subsequent transmission efficiency, and (3) the status of juice transmissibility of the virus. It follows that a "typical" aphid-borne persistent virus would have the following characteristics: (1) it would be retained by the aphid vector for a relatively long period of time, (2) the vector efficiency would not be influenced by a pre-fasting period prior to infection feeding, and (3) it would not be juice transmissible with ordinary facility. A "typical" non-persistent virus would be opposite in these respects. For convenience, it would seem feasible to classify a virus according to present knowledge as being persistent or non-persistent if it had any combination of 2 or more of the 3 characters in common. If such a system proved to be impracticable in the future, then it should be discarded as have been numerous systems of classification in the past.

The valid use of the term latent period in connection with the persistent aphid-borne viruses is of doubtful value. It appears that the term may be justifiable in the transmission concept of certain of the leafhopper transmitted viruses, and possibly also in the case of one or two of the aphid-borne viruses. However, there are now two examples of persistent aphid-borne viruses, *viz.*, the beet-yellows virus (18) and the yellow-net virus which have been relatively intensively studied and which apparently can be transmitted by the vector without the necessary interpolation of a latent period between an infection feeding and a test feeding when previously noninfective insects are used. From this it would seem that the phenomenon of the latent period has less significance than it formerly was judged to have had in being characteristic of the persistent aphid-borne viruses.

It has been assumed in the past that the saliva injected into a plant by the feeding insect is the vehicle for virus transmission by suctorial insects, and it would seem as if some experimental evidence has been presented which would increase the validity of this established view. When an aphid penetrates plant tissues there is a salivary sheath formed, and at the termination of the puncture some probing action occurs until a suitable location is found for the uptake of food. Using this as a basis, it would appear reasonable that if it were possible to force an infective aphid to introduce 10 times as much salivary secretion into a plant as it normally would in a given period of time, there would be a decided increase in the number of infections obtained, if the saliva was transporting the virus. When this was attempted, an increase in the number of infections was obtained. The other factors which could account for such an increase appear to have been reduced to a minimum by the experimental procedure. Thus it would appear that saliva was the vehicle for virus transport.

The next consideration is whether or not the saliva is ejected continuously during feeding, and therefore whether the insect is continuously injecting virus during prolonged feeding periods. It has been common observation that, within limits, prolongation of the test feeding period increases the probability of successful inoculation. The evidence obtained during these studies indicates that the ejection of effective saliva is initial, *i.e.*, during penetration, or shortly after the puncture is terminated. If such is the case, the reason for the beneficial effect in the prolonged feeding periods would be the greater opportunity for a series of punctures. Likewise, the explanation for a positive correlation between the length of the test feeding period and the increase in vector efficiency, until a maximum is reached, is that finally all virus-potent vectors have established infection and consequently prolongation of the test feeding period would be of no avail.

This would seem to reduce the effect of one continuous feeding period by a vector to a minimum, as far as vector efficiency is concerned.

Whether or not the saliva is always contaminated with virus is also a question. The results obtained show that an insect may be spasmodically

active in producing infections, indicating the ejection at times of non-contaminated saliva. This phenomenon has been described in terms of active or passive infectivity. There appears to be no phasic or cyclic occurrence of the active and passive states of infectivity, but the status for want of a better explanation would seem to be determined by individual "potency", a term introduced by Storey (12).

The practical application of such information possibly would be in the explanation of some of the phenomena observed in connection with field spread of virus diseases. There might exist in the field a large, or small, restless infective population, either because of conditions of crowding, in the case of a very large population, or irritation, in the case of a small population, such as might occur during and following the application of insecticides or fungicides, or cultural practices, when the individual would be making large numbers of punctures, but punctures of relatively short duration. In such a case, rapid virus spread might be expected. Also, if such conditions existed, a rate of virus spread might be found which seemingly would be inconsistent with the population density. On the other hand, it is conceivable that a situation could exist where a large but relatively sedentary population existed in the field, thus there would be a relatively small number of penetrations. In such a case the rate of virus spread might be relatively slow in comparison to the population density.

SUMMARY AND CONCLUSIONS

The vector-virus relationships upon which experimental work was done are described. The primary vector of the yellow-net virus of sugar beets appears to be the green peach aphid, *Myzus persicae* (Sulzer), although evidence indicates that *Aphis rumicis* Linn., *Macrosiphum solanifolii* (Ashm.), and *Myzus pseudosolani* Theob., can act as vectors experimentally.

The acquisition threshold was determined to occur at 5 minutes, while the inoculation threshold is approximately 15 minutes after feeding commences, which would seem to indicate that mesophyll penetration is sufficient for acquisition of virus, but the phloem is the essential tissue involved in inoculation. A distinct latent period does not appear to exist.

The green peach aphid retained the virus for 11 days, or for the duration of its life under the experimental conditions. Tests on the effect of multiple feeding punctures indicated that the saliva introduced into the plant in connection with the puncture, *viz.*, the salivary sheath, or the saliva ejected shortly thereafter, is the vehicle used for virus transport. Data presented indicate that virus contamination of the saliva is not continuous, but rather discontinuous. To describe the phases of vector infectivity resulting from the phenomenon, the terms actively and passively infected are introduced.

The vector efficiency of a vector group, composed of 10 apterae, is approximately 75 per cent, while that of a single aptera is approximately 25 per cent.

First instar nymphs, pupae, alate viviparae, and apterous viviparae will transmit the virus, and the virus can be retained through a moult.

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VARIABILITY OF THE CUCURBIT ROOT-ROT FUNGUS, FUSARIUM (HYPOMYCES) SOLANI F. CUCURBITAE[1]

N. PRASAD

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Hypomyces solani Rke. et Berth. emend Snyd. et Hans. is the perfect stage of *Fusarium solani* (Mart.) Appel et Wr. emend Snyd. et Hans.; forms of which cause root rot of several important crops. The form parasitic on squash, *H. solani* f. *cucurbitae* Snyd. et Hans. has been shown by Hansen and Snyder (3) to be an extremely variable fungus. They have found numerous strains in culture and in nature which differ not only in pigmentation, rate and type of growth, but also in their sex and compatibility groups. Some of the strains are more pathogenic than others and are capable of attacking a number of cucurbits including melons and gourds. This study was undertaken to find out if any correlation existed between cultural variation and pathogenicity.

COMPARATIVE MORPHOLOGY OF STRAINS

Thirty-three strains of *Fusarium solani* f. *cucurbitae,* kindly supplied by Dr. H. N. Hansen, were maintained in a pure state on potato-dextrose agar by repeated single conidium transfer at frequent intervals following the method of Hansen and Smith (2).

The thirty-three strains can be grouped into seven distinct groups on the basis of pigmentation, production of sporodochia, amount of aerial mycelium and the type of growth on potato-dextrose agar. The slow, button type of growth is characteristic of strains 22, 25, 26, 28, 32, and 33. The thirty-three strains differ among themselves in pigmentation, from pure white to yellow, green and bluish-green, and in size and septation of the macro-conidia as shown in table 1.

The longest (61.7 μ) occurred in strain 30 and the shortest (20.2 μ) in strain 3. The shape of macro-conidia is one of the characters upon which Snyder and Hansen (4, 5, 6) have based the separation of species in the genus *Fusarium.* Macro-conidia of *F. solani* may have pointed or blunt ends and the degree of curvature varies. The macro-conidia of strain 3 show blunt ends and only slight curvature in contrast to the other strains. Anastomosis of the conidia *in situ* is characteristic of strains 7 and 19.

If one were to follow Wollenweber and Reinking's (7) classification of the Fusaria, based mostly on the size of macro-conidia, one could separate the thirty-three strains into five or six different species. This study shows that neither size of conidia nor a reasonable variation in shape can be relied upon as a morphological character in the determination of species in

[1] The work was done under the guidance of Professor H. N. Hansen in partial fulfillment of the requirements for the Ph.D. degree. The writer is extremely indebted to him and Professor W. C. Snyder for the help he received from them during the course of these investigations.

Fusarium. Were every variation in conidial size or in other cultural character used to distinguish species in this pathogen, the number of species causing squash root rot would be in proportion to the number of variants recovered. But since all 33 of the strains studied have been derived from the perithecial stage (*Hypomyces*) of one fungus, it would seem more logical to recognize as the normal variability of that fungus all variations in cultural characters displayed by these 33 strains or by any additional ones so derived. Some of the cultural variations are evident in figure 1.

TABLE 1.—*Range in microns of 50 macro-conidia taken at random from each of 33 different strains of Fusarium solani f. cucurbitae in cultures 3 weeks old*

Strain	Length	Width	No. of Septa
1	39.2–51.3	3.0–4.2	1–4
2	31.3–46.74	3.5–4.5	1–4
3	20.2–30.76	3.0–3.8	1–2
4	35.2–53.2	3.5–4.5	0–4
5	36.5–52.6	3.9–5.2	0–5
6	30.5–42.6	4.0–5.2	0–4
7	34.3–45.0	3.8–5.2	0–4
8	35.0–60.3	4.1–5.8	0–5
9	33.2–40.4	3.8–4.6	0–3
10	38.7–50.5	3.7–5.1	0–4
11	40.1–55.6	3.9–5.4	0–5
12	39.2–57.6	3.9–5.4	0–5
13	31.2–46.2	3.6–4.7	0–4
14	42.7–61.3	4.1–5.8	0–5
15	40.7–61.5	4.0–5.8	0–5
16	30.6–41.5	3.9–4.6	0–4
17	42.5–67.5	3.7–5.8	0–6
18	30.5–45.4	3.5–4.2	0–4
19	34.3–51.2	3.9–5.1	1–4
20	29.3–43.6	3.6–4.8	0–4
21	31.2–47.4	3.2–4.9	1–4
22	36.7–51.2	3.5–4.9	1–4
23	34.2–55.2	3.9–4.2	0–4
24	31.3–48.4	3.5–4.9	0–5
25	37.2–55.4	3.8–5.4	0–5
26	31.2–46.3	3.2–4.7	0–4
27	27.5–36.7	3.5–4.8	0–3
28	35.6–48.5	3.9–4.7	1–4
29	37.0–49.2	3.8–4.9	1–4
30	41.2–61.7	3.9–5.7	1–5
31	36.7–49.2	3.5–4.9	1–4
32	20.3–35.3	3.3–4.7	0–2
33	37.8–57.3	3.7–5.7	1–5

COMPARATIVE PATHOGENICITY OF STRAINS

Experiments to test the relative pathogenicity of these strains were carried out in sterilized soil in the greenhouse. Two methods of inoculation were followed. The first method consisted in dipping squash seed (Black Zucchini, *Cucurbita pepo L.*) in a spore suspension of each of the thirty-three strains of *Fusarium solani*. f. *cucurbitae* and planting in 6-inch pots In the second method of inoculation the seeds of squash were sown in pots and after the seedlings had emerged, spore suspensions from the different

strains were poured into the pots. To prevent contamination from one pot to another by splashing of water, the pots were sub-irrigated.

With virulent strains, the symptoms of infection appeared in about a week's time. The first symptom was the appearance of discoloration at the foot which proceeded upward in the form of a streak in the cortical region. The plants died within two days. With weakly parasitic strains, the plants were not killed even ten days after the appearance of first symptoms. On the basis of number of days required to kill the plants and the extent of the lesions produced, the strains can be grouped as very

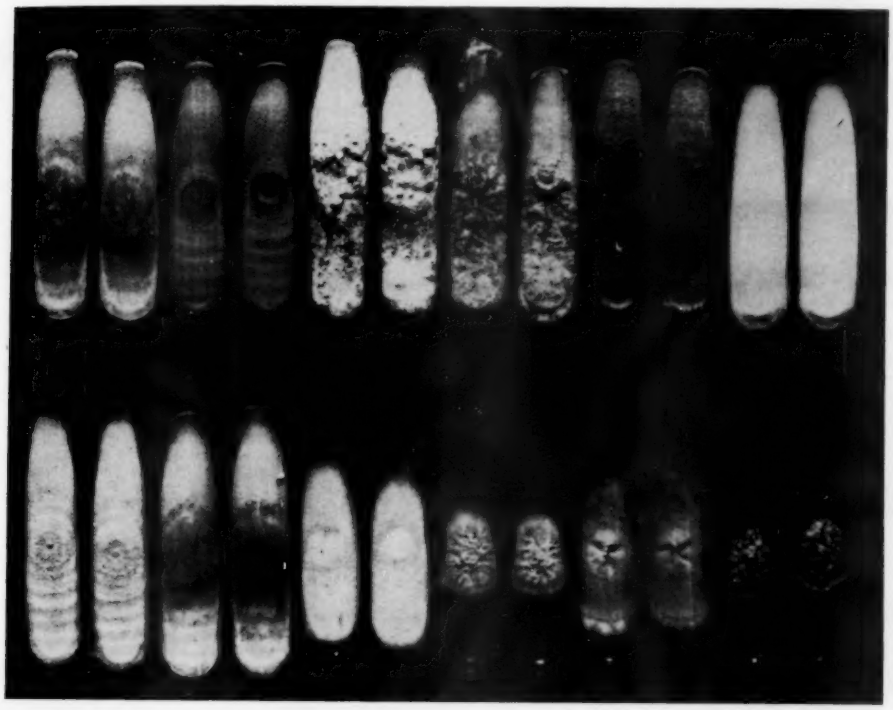

FIG. 1. Some of the strains of *Fusarium solani* f. *cucurbitae* showing variation in the rate and type of growth and in pigmentation. All cultures were started from single conidia and are of the same age and grown on the same medium.

virulent, virulent, fairly virulent, weak, very weak, and non-parasitic. The strains 8, 12, and 19 are very virulent and killed the plants in 10 days. Eighteen came under the category of virulent strains as they caused the death of the plants in 12 to 15 days. There was only one strain, 33, which could be called fairly virulent as it caused lesions in 10 days. Weak strains 22, 28, 29 caused lesions in 15 days. Strains 25 and 26 were classed as very weak pathogens because only very slight lesions appeared after 20 days. Strain 32 was the only one which was non-parasitic under the conditions of the experiments since it failed to produce lesions of any kind even 30 days after inoculation.

RELATION OF PATHOGENICITY TO GROWTH RATE

To find out whether rate of growth had any relationship to pathogenicity, the five strains, 8, 12, 22, 25, and 33, were grown in U tubes designed by H. N. Hansen, each consisting of 2-cm. glass tubing, bent in a U fashion, with each arm 30 cm. in length and the ends bent upwards. These tubes were half-filled with potato-dextrose agar, sterilized, and cooled in a horizontal position (Fig. 2). They were inoculated at each end with a single spore, a different strain being used for each tube. After a few days the margin of growth was marked on each tube and measurements of subsequent

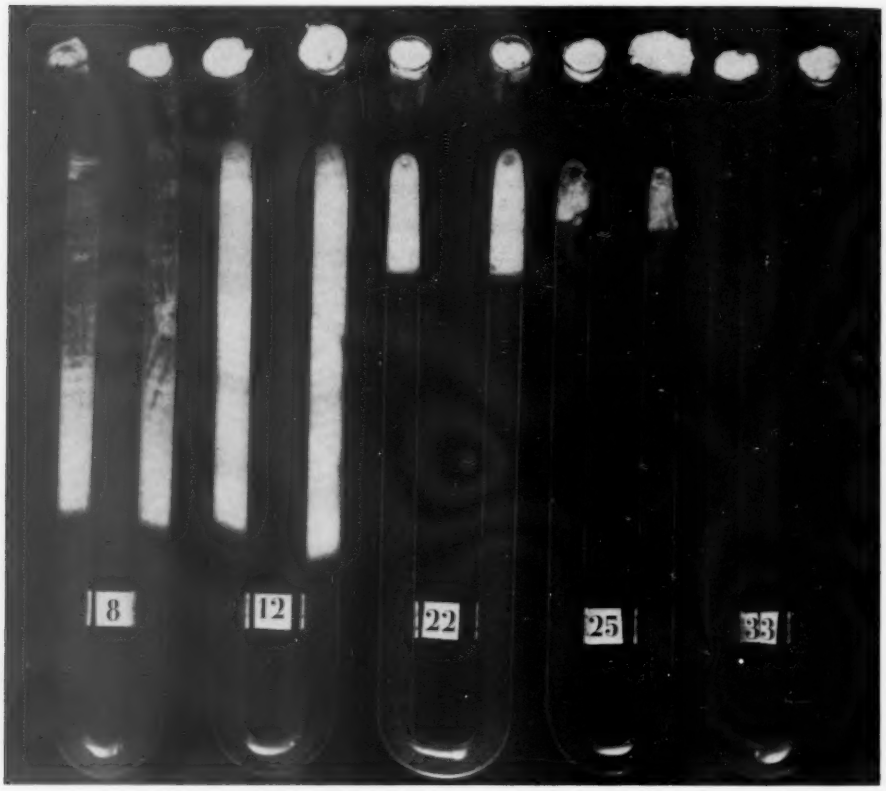

FIG. 2. Comparative rates of growth of strains 8, 12, 22, 25, and 33 when cultured simultaneously in U-tubes under laboratory conditions. Strains 8 and 12 are the most rapid in growth and are the most virulent.

growth were taken from this line every day (Table 2). Strains 8 and 12 grow most rapidly and almost at the same rate and are very virulent. Strains 33 and 25 have the slowest rate of growth while strain 22 is intermediate and these 3 strains are less virulent than 8 and 12.

An infection experiment was conducted to find out if there was any closer correlation between rate of growth and pathogenicity. Seedlings were grown in pots and when they were about 3 inches tall, a spore suspension of the pathogen was poured into each pot. Spores from one culture tube

TABLE 2.—*Comparative rates of radial growth in millimeters of certain strains of Fusarium solani f. cucurbitae at room temperature in U-tubes*

Strain	Total growth in 2 weeks, mm.	Average rate of growth per day, mm.
8	86.8	6.2
12	88.0	6.3
22	23.8	1.7
25	17.0	0.5
33	10.0	0.4

where the fungus had grown for 3 weeks, were used for infecting one pot. The opaqueness of the spore suspensions was comparable in each case.

The results obtained (Table 3) show that the rapidly growing strains (8 and 12) are more pathogenic than 22, 25, and 33 which grow slowly. But strain 33 which grows at a slower rate than either 22 or 25 is more pathogenic than 22 or 25.

The comparison of the pathogenicity of different strains in this manner is open to criticism since it was not certain that exactly the same amount of inoculum was used in each case. A better method of comparison would be to bring about infection with a single spore of each strain and to measure the size of the lesions produced by each in a given period of time. This method would give a measure of the virulence of a strain in terms of time.

Single spore inoculation.—Single germinated spores were picked from water agar plates as used in the preparation of single spore cultures (5). The pieces of agar, measuring 1 to 2 mm. on a side, bearing single germinated spores were applied to the stems of potted squash seedlings. It was found that the pieces of agar dried up very quickly and that in the greenhouse infection could not be established by this method without some modification. It was necessary to keep the plants in a moist chamber long enough for the fungus to establish itself. It was found that with the fast growing strains, symptoms appeared by the eighth day after inoculation. However no symptoms appeared during this period where the slow-growing strains were tried.

In another test, seedlings were grown from sterilized seed in Petri dishes on moist filter paper sterilized by boiling. Six days after germination, a

TABLE 3.—*Rate of killing of squash plants when pots of seedlings were inoculated separately with strains 8, 12, 22, 25, and 33 of Fusarium solani f. cucurbitae, using comparable amounts of inoculum*

Strain of fungus	Total number of plants killed	Time required to kill the plants	General remarks
8	5	9 days	Plants completely rotted
12	5	9 days	Plants completely rotted
22	none	.....	Lesions produced at the foot
25	none	.....	Very slight lesions
33	1	15 days	Lesions well marked

small piece of agar containing a single germinated spore was placed on each seedling. When the seedlings became too big for the Petri dishes, they were transferred to small Ehrlenmeyer flasks containing Hoagland's solution which was changed after every 4 days to prevent the growth of algae in the flasks. Five squash seedlings were inoculated with single germinated spores from each of four strains 8, 12, 22, and 33. The extent of the lesions produced by each strain was measured 6 days after inoculation (Table 4). Strains 8 and 12 produced the largest lesions, 15×5 mm. in size. The lesions caused by strain 33 were much greater than those caused by strain 22 although the latter had a higher rate of growth than the former. The lesions produced by these two strains measured 5×2 and 2×1 mm., respectively. Seventeen days after inoculation the plants inoculated with strain 33 died, while strain 22 did not kill the plants.

From the above it appears that there is no consistent correlation between the rate of growth of the fungus and its pathogenicity. Strains 8 and 12

TABLE 4.—*Comparison of pathogenicity of strains 8, 12, 22, and 33 of Fusarium solani f. cucurbitae obtained by inoculating squash plants with a single spore from each strain*

Strain	Rate of mycelial growth (in mm.) per day	Size of the lesion (in mm.) six days after inoculation	Time (in days) required to kill the plant
8	6.2	15×5	10
12	6.3	14×5	10
22	1.7	2×1	No death
33	0.4	5×2	17

are the fastest growing strains and the most virulent but strain 22, which grows more rapidly than 33, is not so pathogenic as 33.

NUTRITIONAL REQUIREMENTS OF CERTAIN STRAINS

Beadle and Tatum (1) found that single spore strains of *Neurospora crassa* Shear et Dodge differed in nutritional requirements. Their work suggested that by providing proper vitamins to the different strains of *Fusarium solani* f. *cucurbitae*, it might be possible to make the slow-growing strains grow faster and show greater pathogenicity.

With that object in view, certain strains of *Fusarium solani* f. *cucurbitae* were grown on Beadle's (1) minimal medium and on potato-dextrose agar (P.D.A.) to which the following substances were added separately: thiamin, niacin, folic acid, pyridoxine, pantothenic acid, and biotin. The amount of vitamins added per liter was as follows: thiamin 100 mg, niacin 200 mg, pyridoxine 50 mg, folic acid 15 mg, pantothenic acid 200 mg, and biotin 6×10^{-6} gm. These are the concentrations used by Beadle. Equal quantities of each of the above media were poured into Petri dishes and each of these was inoculated with a single spore of strains 8, 22, 25, and 33. A line was marked with a wax pencil across each Petri dish and it was on

this line that the measurement of the diameter of each colony was taken daily for a period of two weeks (Table 5).

The results show that *Fusarium solani* f. *cucurbitae* does not grow well on synthetic media even in the presence of various vitamins. Growth of all the strains was much better on P.D.A. than on Beadle's medium.

Strain 8 normally grows well on P.D.A. and produces aerial mycelium and green pigmentation. It failed to produce any aerial mycelium or pigmentation when grown on Beadle's medium plus the vitamins. Strains 22 and 25 did not grow well on any one of the media, but 22 grew much better than 25. Strain 33 grew poorly on all media with the exception of P.D.A. to which 5×10^{-6} gm. per liter of biotin had been added.

TABLE 5.—*Growth of four strains of Fusarium solani f. cucurbitae on media containing different growth substances*

Medium	Average rate of growth of strains (in mm. per day)				Total growth (in mm.) of strain after 2 weeks			
	8	22	25	33	8	22	25	33
Beadle's minimal medium	2.0	0.5	0.2	0.2	28.0	7.0	2.8	2.8
Beadle's minimal medium + Thiamin	2.0	0.4	0.2	0.2	28.0	5.5	2.8	2.8
Beadle's minimal medium + Niacin	2.2	0.6	0.2	0.2	30.0	8.4	2.8	2.8
Beadle's medium + Pantothenic acid	2.1	0.5	0.15	0.13	29.0	7.0	2.0	1.8
Beadle's medium + Biotin	2.3	0.5	0.2	0.3	32.0	7.0	2.8	4.7
Beadle's medium + Pyridoxine	2.0	0.4	0.2	0.2	28.0	5.5	2.8	2.8
Beadle's medium + Folic acid	2.2	0.5	0.25	0.2	30.0	7.0	3.5	2.8
Potato-dextrose agar	6.3	1.7	0.5	0.4	88.0	24.0	7.0	8.0
Potato-dextrose agar + Thiamin	6.2	1.7	0.6	0.5	87.0	24.0	8.5	7.0
Potato-dextrose agar + Niacin	6.2	1.8	0.55	0.6	87.0	25.0	7.7	8.5
Potato-dextrose agar + Pantothenic acid	6.4	1.8	0.5	0.5	89.6	25.0	7.0	7.0
Potato-dextrose agar + Biotin	6.3	1.7	0.6	2.3	88.0	24.0	8.5	32.2
Potato-dextrose agar + Pyridoxine	6.2	1.6	0.5	0.4	87.0	22.0	7.0	5.5
Potato-dextrose agar + Folic acid	6.2	1.7	0.4	0.5	87.0	24.0	5.5	7.0

Normally, it grows very slowly in the form of a button on P.D.A., producing a bluish pigmentation and as it grows the medium becomes colored deep brown. But, when it was grown on P.D.A. to which biotin was added, instead of the characteristic button type of growth, it produced an increased amount of mycelium and resembled strain 8. The medium retained its semi-transparent nature. This experiment suggested that strain 33 required biotin for its normal growth and that the amount of biotin ordinarily present in P.D.A. was not sufficient for it to grow normally.

EFFECT OF BIOTIN ON PATHOGENICITY

The previous experiment suggested that biotin might increase the pathogenicity of strain 33 since an additional supply of biotin stimulated its growth in vitro. Squash seedlings grown in Petri dishes were inoculated with single germinated spores from strains 8 and 33, and after inoculation

were transferred to Ehrlenmeyer flasks containing Hoagland's solution. In one series of flasks, biotin was added at the rate of 10×10^{-6} gm. per liter of Hoagland's solution. It was observed that strain 33 was more pathogenic in the medium which contained the additional supply of biotin. The extent of lesions produced by strain 33 in the presence of the additional biotin, was much greater than in the absence of biotin. Although the addition of biotin increased the pathogenicity of strain 33, it was still less pathogenic than strain 8. This difference might be due to the inability of strain 33 to produce something else which plays as important a role as biotin.

DISCUSSION

The study of variation in size of macro-conidia within a single form of one species based on direct descendency, shows clearly that size of conidia is not a reliable character for the identification of species of *Fusarium*. If one were to base species on the measurement of conidia, it would be necessary to place the thirty-three strains of a single species into at least five or six different species. Wollenweber and Reinking (7) in their classification of Fusaria, have stressed the importance of size of macro-conidia and production of pigment among other characters. Evidence presented here indicates that these characters are not dependable for the identification of Fusaria. These studies support Snyder and Hansen's (4, 5, 6) system of classification of Fusaria wherein they do not base their species on the measurement of macro-conidia but regard the differences in measurements as normal variation between clones of the same species. One is convinced of the usefulness of their system of classification when one examines the variation in size and in production of sporodochia, sclerotia, and pigment within the thirty-three strains of *F. solani* f. *cucurbitae*.

Since different strains of *Fusarium solani* f. *cucurbitae* have different rates of growth, one is tempted to correlate pathogenicity with rate of growth. Strains which have a higher rate of growth should be more pathogenic than the slow growing ones. Strains 8 and 12 grow fastest and are also most pathogenic. But this has not been found to be true when other strains are compared for pathogenicity. Strain 33 which grows at a slower rate than strains 22, 25, 26, or 28, has been found to be more pathogenic. This behavior was more apparent with single spore inoculations. This method of inoculation has proved to be a very reliable and practical method of comparing the different strains of *F. solani* f. *cucurbitae* for pathogenicity.

SUMMARY

Thirty-three strains of *Fusarium solani* f. *cucurbitae* were found to differ from each other in culture type, rate of growth, pigmentation and size of macro-conidia.

Inoculation of the host plant (squash seedling) with a single germinated spore provided a satisfactory method for the comparison of different strains

with respect to pathogenicity. The strains were found to vary greatly in pathogenicity but consistent correlation was not found between rate of growth and pathogenicity. Strains 8 and 12 are the fastest growing strains and also the most highly pathogenic. Strain 33 which has a slower rate of growth than strains 22 and 25, was found to be more pathogenic than either strain 22 or 25.

Rate of growth of strain 33 was increased with additional amounts of biotin in the medium. It also showed greater pathogenicity in the presence of an additional supply of biotin.

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THE OCCURRENCE OF PUCCINIA CORONATA ON RHAMNUS FRANGULA IN CANADA[1]

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Crown rust (*Puccinia coronata* Cda.) has been studied extensively in both Europe and America. These studies have shown that this rust is composed of a large number of morphologically similar strains or varieties that have their aecial stages on several species of the Rhamnaceae and Elaeagnaceae and their uredial and telial stages on various grasses. In Europe, the aecial stages of crown rust varieties occur most commonly either on *Rhamnus cathartica* L. or *R. Frangula* L. A number of the European crown rust varieties that occur on *R. cathartica* apparently also occur in America. However, none of those that occur on *R. Frangula* has hitherto been reported to occur on that host in North America, although the aecial host grows commonly in many localities in the United States and Canada.

Since 1939, aecial infections of a variety of crown rust have been collected every spring on *Rhamnus Frangula* at Fredericton, N. B. Telial infections of this rust have also been collected in three different years on *Agrostis* spp. growing in the vicinity of the infected *R. Frangula* bushes. This rust has been cultured on its aecial host, *R. Frangula,* and on several species of its gramineous host under greenhouse conditions at the Dominion Laboratory of Plant Pathology, Winnipeg. A description of this crown rust variety is given in the present paper.

LITERATURE REVIEW

The heteroecious nature of crown rust was demonstrated in 1865 by De Bary (5), who infected the leaves of two European species of *Rhamnus, R. Frangula* and *R. cathartica,* with teliospores of crown rust collected on grasses.

In 1874, Nielsen (18) obtained heavy uredial infection on *Lolium perenne* L. with aeciospores from *Rhamnus cathartica,* and with the urediospores thus obtained on this grass he infected cultivated oats. He could not, however, cause infection on *L. perenne* with the aeciospores that occurred on *R. Frangula* and, therefore, came to the conclusion that two rusts were included under the name *Puccinia coronata.* To the rust that had its aecial stage on *R. cathartica* and its uredial and telial stages on *L. perenne* and cultivated oats he gave the name *P. Lolii* Nielsen.

Experiments carried out by Plowright (19) in 1889 further demonstrated the difference between the varieties of crown rust that had their

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aecial stages respectively on *Rhamnus cathartica* and *R. Frangula*. He considered that two species of crown rust were being confounded under the name *Puccinia coronata*.

In 1894, Klebahn (10) found that the crown rusts occurring on *Avena sativa* L., *Arrenatherum elatius* (L.) Mert. and Koch, and *Lolium perenne* could infect *Rhamnus cathartica* but not *R. Frangula*. Klebahn, like Nielsen and Plowright, came to the conclusion that there were two crown rust species present and, to the rust on *R. Frangula*, he gave the name *Puccinia coronata* (Corda) Kleb. The rust on *R. cathartica* he named *P. coronifera* Kleb. He was apparently unwilling to accept the name *P. Lolii* Niels. that Nielsen had previously given to this rust.

Eriksson (8) and Klebahn (11, 12) found that the crown rusts were highly specialized in respect to their gramineous hosts and listed several varieties (*formae speciales*) of each rust. Their final arrangement of the varieties listed was as follows:

Puccinia coronata with aecia on *Rhamnus Frangula*

1. var. *Agropyri* Erikss.—on *Agropyron repens* (L.) Beauv.
2. var. *Agrostis* Erikss.—on *Agrostis stolonifera* L. and *A. vulgaris* With.
3. var. *Calamagrostis* Erikss.—on *Calamagrostis lanceolata* Roth.
4. var. *Holci* Kleb.—on *Holcus mollis* L. and *H. lanatus* L.
5. var. *Phalaris* Kleb.—on *Phalaris arundinacea* Roth.

Puccinia coronifera with aecia on *R. cathartica*

1. var. *Avenae* Erikss.—on *Avena sativa* L.
2. var. *Alopecuri* Erikss.—on *Alopecurus pratensis* L.
3. var. *Agropyri* Erikss.—on *Agropyron repens* (L.) Beauv. only.
4. var. *Epigaei* Erikss.—on *Calamagrostis epigeios* (L.) Roth.
5. var. *Festucae* Erikss.—on *Festuca elatior* L. and *F. gigantea* (L.) Vill.
6. var. *Glyceriae* Erikss.—on *Glyceria aquatica* Wahlb.
7. var. *Holci* Kleb.—on *Holcus lanatus* L. only.
8. var. *Lolii* Erikss.—on *Lolium perenne* L.

Muehlethaler (15, 16), in 1911, added to this latter group a ninth variety, *Bromi*, which occurs on several species of *Bromus;* and Brown (3), in 1947, isolated and described the variety *Arrhenatheri*, which was collected on *Arrhenatherum avenaceum* Beauv.

In America, Arthur and Holway (2) in 1898, and Carleton (4) in 1899 succeeded in producing aecial infections on the leaves of *Rhamnus lanceolata* Pursh., a common native species of the southern United States. They made their inoculations with teliospores from cultivated oats. Later, Arthur (1) obtained aecial infection on the leaves of *R. alnifolia* L'Her., a northern native *Rhamnus* species, with teliospores from *Calamagrostis canadensis* (Michx.) Beauv. Both Arthur and Carleton disagreed with Nielsen's and with Klebahn's nomenclature of crown rust. They doubted the existence, at least in America, of two crown rust species, and therefore referred to the crown rust occurring in America as *Puccinia coronata* Cda.

Melhus and Durell (13) found, in 1915, that teliospores on oats could cause heavy aecial infections on *Rhamnus cathartica* but none on *R. Frangula.*

Melhus et al. (14) tested several species of *Rhamnus* in 1919, 1920, and 1921, to crown rust occurring on oats, *Calamagrostis canadensis,* and *Festuca elatior.* In these tests, *R. Frangula* proved to be highly resistant to the rust on all these gramineous hosts.

Deitz (6, 7), working at Ames, Iowa, obtained in a few of his trials several pycnial infections, but never aecial infections, on *Rhamnus Frangula* when inoculations were made with teliospores from oats. He kept under observation for the 8-year period, 1916 to 1924, a number of bushes of *R. Frangula* growing on the campus at Ames, but at no time found pycnia or aecia on these bushes, although bushes of *R. cathartica* growing nearby became infected each year. In 1923 and 1924, he kept also under observation ten plantings of *R. Frangula* in the New England States. No aecia were found on these plantings, whereas aecial infections were abundant on *R. cathartica* in that area. In experiments carried out at Ames, Iowa, in 1929, Murphy (17) failed to obtain infection on *R. Frangula* with teliospores from oats. In these same tests he obtained normal spermogonia and aecia on *R. cathartica* with the teliospore material that failed to cause infection on *R. Frangula.*

Fraser and Ledingham (9), in 1933, collected and studied four distinct varieties of crown rust at Saskatoon, Saskatchewan, Canada. These were as follows:

Var. *Avenae*—with aecia on *Rhamnus cathartica.*

Var. *Calamagrostis*—with aecia on *R. alnifolia.*

Var. *Elaeagni*—with aecia on *Elaeagnus commutata* Bernh.

Var. *Bromi*—with aecia on *Lepargyraea canadensis* (L.). Greene.

The variety *Bromi* described by Fraser and Ledingham failed to infect *Rhamnus cathartica* and evidently was distinct from the variety *Bromi* described by Muehlethaler in Europe. Fraser kept under observation for several years specimens of *R. Frangula* growing at Saskatoon, but failed to find any rust infections on that host.

EXPERIMENTAL WORK

Heavy aecial infections were observed on *Rhamnus Frangula* at Fredericton, N. B., in 1939 by Mr. S. F. Clarkson and Mr. J. L. Howatt, of the Dominion Laboratory of Plant Pathology, Fredericton, N. B. So far as the author has been able to ascertain, this was the first recorded observation of the occurrence of aecial infection on *R. Frangula* in America and, to date aecial infection on this host has not been reported elsewhere in North America. This rust has been observed and collected on *R. Frangula* at Fredericton every year since 1939.

A search was made in the vicinity of the infected buckthorn bushes for the telial stage of the rust occurring on them and abundant telial infections

of a crown rust were found nearby on a grass identified as an *Agrostis* sp. Several collections of the teliospores occurring on this grass and of the aecia occurring on *Rhamnus Frangula* were sent to the Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba for further study.

In the tests that were made, the aeciospores from the infections on *Rhamnus Frangula* failed to cause infection on cultivated oats but normally infected seedling plants of several species of *Agrostis*. Coronate teliospores were formed on the grass hosts in the greenhouse. These teliospores, when used for the inoculation of *R. Frangula*, caused normal pycnia and aecia to form. The teliospores found on *Agrostis* in the vicinity of the infected

TABLE 1.—*The reactions of oats and 19 grass species to the rust occurring on Rhamnus Frangula*

Host	Rust reaction
Agrostis hyemalis Walt.	Susceptible
A. tenuis Sibth. (*A. vulgaris* With.)	do
A. lacnantha Nees.	do
A. nebulosa Boiss. and Rent.	do
A. rupestris Champ.	do
A. stolonifera L.	Moderately susceptible
Calamagrostis canadensis (Michx.) Beauv.	Moderately resistant
C. arundinacea Roth.	do
Agrostis alba L.	Immune
A. spica-venti L.	do
A. verticillata Vill.	do
A. canina L.	do
Agropyron repens (L.) Beauv.	do
Avena sativa L. (Victory)	do
Calamagrostis lanceolata Roth.	do
Dactylis glomerata L.	do
Holcus lanatus L.	do
Lolium multiflorum Lam.	do
L. perenne L.	do
Phalaris arundinacea L.	do

bushes of *R. Frangula* germinated readily and caused heavy aecial production on *R. Frangula*, but failed to infect *R. cathartica*.

Cultivated oats and a number of grasses were inoculated in the seedling stage with aeciospores from *Rhamnus Frangula*. Each species investigated was subjected to several different inoculation tests with aeciospores. Later, the oats and grasses were all inoculated with the urediospores that resulted on the susceptible grasses from the infections caused by the aeciospores. Table 1 gives the rust reactions of the grass species tested to the rust occurring on *R. Frangula*.

The behavior of this rust on the grass hosts tested shows it to be identical with a variety of crown rust that Eriksson (8) isolated in Europe in 1897 and named *Puccinia coronata* Corda forma *Agrostis* Erikss. The rust variety isolated by Eriksson, like the one under study, had its aecial stage on *R. Frangula* and its uredial and telial stages on *Agrostis vulgaris* (*A. tenuis*), *A. stolonifera*, and other grasses.

The aecia on *Rhamnus Frangula* occur on the petioles and on the under

side of the leaves, with the majority of the aecia confined to the leaves. The aecial pustules vary from round to oval, according to their location on the leaves. The ones that form between the leaf veins are usually small and circular, whereas those that form on the leaf veins are larger and oval, their oval shape being due to the tendency of the pustules to spread along the leaf veins. Some hypertrophy of the host tissues is associated with aecial formation, particularly when the aecia occur on the leaf petioles. Measurements were taken of aecial cups formed outdoors at Fredericton in 1947. The average external diameter of the 120 cups measured was 247 microns, and they ranged in size from 197 to 329 microns in external diameter.

Measurements were made of the aeciospores, urediospores, and teliospores of *Puccinia coronata* var. *Agrostis*. The aeciospores measured were taken from field collections obtained at Fredericton, N. B., in 1947, and the urediospores and teliospores measured were formed on *Agrostis tenuis* in the greenhouse at Winnipeg in 1947. In measuring the aeciospores and

TABLE 2.—*The minimum, maximum, and mean length and width of aeciospores, urediospores, and teliospores of Puccinia coronata var. Agrostis*

Spore form	Spore length in microns			Spore width in microns			No. of spores measured
	Minimum	Maximum	Average	Minimum	Maximum	Average	
Aeciospore	15.5	25.7	20.1	13.3	20.4	17.3	100
Urediospore	17.7	26.6	21.2	14.2	25.7	19.5	100
Teliospore	31.5	60.9	40.8	12.6	23.1	16.8	200

urediospores, the greater spore diameter was taken as the spore length, and the lesser spore diameter as the spore width. The teliospores were measured for length from the attachment of the pedicel to the exterior of the apex of the upper cells. Their width was measured across the apex of the upper cell. The spore measurements are in table 2.

The average spore sizes recorded in table 2 for aeciospores, urediospores, and teliospores are almost the same as the spore sizes recorded by Fraser and Ledingham (9) for the crown rust variety that they designated as *Puccinia coronata* var. *Bromi*, but are definitely smaller than the spore sizes recorded by these authors for *P. coronata* var. *Avenae* and *P. coronata* var. *Elaeagni*.

The average length and width of the teliospores of *Puccinia coronata* var. *Agrostis*, as given by Eriksson (8), agree very closely with those of the rust under study. However, the range in spore length (38–44 μ) recorded by Eriksson was much less than that shown in table 2.

The teliospores varied greatly in size and shape, as is shown in figure 1.

DISCUSSION

Although *Puccinia coronata* var. *Agrostis* has not been found on its aecial host anywhere in America, except at Fredericton, N. B., it probably

occurs in the uredial and telial stages on its grass hosts elsewhere in America. Mr. I. L. Conners, Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, states that three collections of crown rust on *Agrostis stolonifera*—one from Kentville, N. S., and two from near Chelsea, Que.—are now in the Mycological Herbarium at Ottawa. Freeman Weiss (20) has recorded the occurrence of a crown rust on *A. alba* L., *A. canina* L., *A. diegoensis* Vasey, *A. exarata* Trin., *A. palustris* Huds., and *A. tenuis* in many localities in the United States. The exact identity of the variety or varieties of crown rust occurring on these grass hosts cannot, however, be established until the appropriate aecial-host inoculation tests have been carried out.

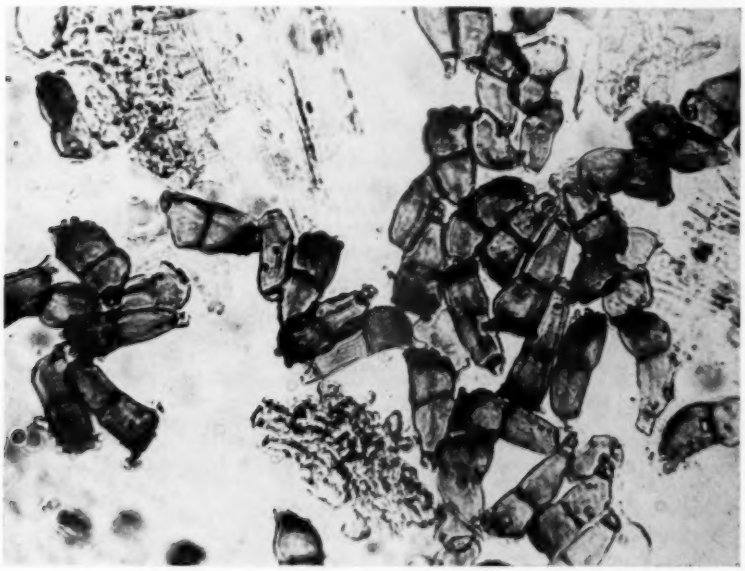

FIG. 1. Teliospores of *Puccinia coronata* var. *Agrostis* (×350).

Since the aecial host of this rust, *Rhamnus Frangula*, as well as several of its uredial and telial hosts, *Agrostis* spp., are native European species, it is probable that the rust has been introduced into America from Europe.

SUMMARY

The aecial stage of a European crown rust, *Puccinia coronata* var. *Agrostis*, has been collected on *R. Frangula* at Fredericton, N.B. each year since 1939.

The telial and uredial stages of this rust were collected on *Agrostis* spp. in 1945, 1946, and 1947, growing in the vicinity of infected bushes of *Rhamnus Frangula*.

This rust can infect some, but not all, species of *Agrostis*.

Aeciospores, urediospores, and teliospores of *Puccinia coronata* var. *Agrostis* are somewhat smaller than the corresponding spores of *P. coronata*

var. *Avenae*, but are almost the same size as those of some other varieties of crown rust.

To date, aecial infections have not been reported on *Rhamnus Frangula* anywhere in North America, except at Fredericton, N. B.

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SYNCHYTRIUM FOUND ON THE ROOTS OF STRAWBERRY[1]

S. M. Zeller and Leo Campbell

(Accepted for publication October 16, 1948)

In March, 1946, small galls were discovered on the roots of Marshall strawberry plants grown near Lynden, Whatcom County, Washington. Inspectors first thought these to be caused by root-knot nematode but an expert nematologist found no parasitic nemas present. When some of the galls were crushed, however, sori of a *Synchytrium* were discovered.

The root galls are mostly less than 1 mm. in diameter and 2–4 mm. long. They may be lateral growths on rootlets or when within the rootlet itself they usually distort the tissues, forming a hook-shaped gall.

Normal growth of strawberry plants does not seem to be affected by the infection.

The fungus sori for the most part are on the inner curve of the hooked galls or on the surface of the gall next to the rootlet from which a lateral gall is produced.

The sori are rather bright yellow and when crushed may be seen to possess several sporangia. The sporangia are rather thin-walled, about 50–60 × 60–80 μ in diameter. At the time observed most of the sporangia were full of swarm spores which were very motile when liberated. The swarm spores are spherical, uniciliate, uninucleate, and about 1.5–2 μ in diameter.

No *Synchytrium* has been reported on the roots of strawberry. Seymour[2] lists *Synchytrium globosum* on *Fragaria* (under *Pycnochytrium*), and *S. aureum* on *Fragaria virginiana*. Dr. Freeman Weiss of the U. S. Plant Disease Survey writes that all of the material of these two species in the Mycological Collections at Beltsville "consists of leaves or stems and these are the host parts that are characteristically infected by *Synchytrium* spp. In fact, I do not know of any record of Synchytrium galls on roots. . . . So, your Synchytrium gall on strawberry roots appears to be a novelty indeed."

Whether this organism was introduced into this particular strawberry field through some previous crop is not known. The land was first cleared in 1939 of Douglas fir, alder, etc., and planted to strawberries that year and the following three years. In 1943 and 1944 there were green manure crops of rye and vetch, and red clover, and then strawberries again in 1945.

[1] Published as a Technical Paper with the approval of the Directors of the Oregon Agricultural Experiment Station and Washington Agricultural Experiment Stations. Technical Paper No. 540 from the Oregon Station, and Scientific Paper No. 793, College of Agriculture and Agricultural Experiment Stations, State College of Washington. Contribution from Plant Pathology.

[2] Seymour, A. B. Host Index of the Fungi of North America. Cambridge, Mass. 1929. (See p. 387.)

We believe this *Synchytrium* differs from other described species and therefore is presented here as **Synchytrium fragariae** sp. nov.

Soris multisporangiatibus, contentu flavo; zoosporangiis tenuo-muriatis, ellipsoideis vel subglobosis, vel subcuboideis, 50–60 × 60–80 μ crassis, e zoosporam maturitas faretis; zoosporis sphaeroideis, 1.5–2 μ crassis, 1-ciliatis, uninucleatis.

In gallis noduloideis in radicis parvis Fragariae, prope Lynden, Washington, Amer. bor.

Sorus multisporangiate, light yellowish; zoosporangia thin-walled, ellipsoid to subglobose, sometimes with flattened sides just after liberation, 50–60 × 60–80 μ in diameter, filled with swarm spores at maturity; swarm spores spherical, 1.5–2 μ in diameter, uniciliate, uninucleate.

Type locality: Near Lynden, Washington.

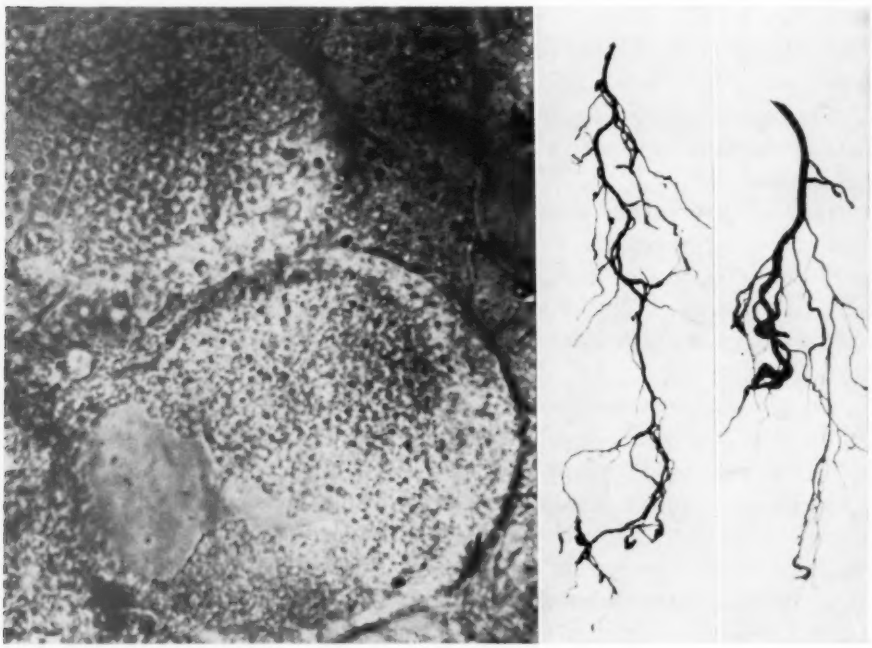

FIG. 1. Synchytrium on strawberry roots. Right, Strawberry roots with galls caused by *Synchytrium fragariae*, × 1; left, zoosporangia showing contents of zoospores (swarm spores), × 1000.

Habitat: In tiny nodule-like galls on roots of cultivated strawberry (*Fragaria*).

Distribution: Known from the type locality only, but by correspondence Dr. Gerald Thorne, Nematologist, U. S. Department of Agriculture, stationed at Salt Lake City, Utah, says the galls are "apparently the same as I found on some material from northern California several years ago," and the cause of which was not known. In that case the causal organism was not seen.

SUMMARY

A new species of *Synchytrium* found to cause tiny, nodule-like galls on the roots of cultivated strawberry (*Fragaria*), is described as *Synchytrium fragariae*. The type locality is near Lynden, Whatcom County, Washington.

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APPLICATION OF THE BACTERIOPHAGE–LYSIS TECHNIQUE FOR THE IDENTIFICATION OF PLANT PATHOGENIC BACTERIA

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(Accepted for publication October 18, 1948)

The specificity of bacteriophage lysis and the simplicity of the procedure for detecting lysis affords a practical means by which to identify bacterial plant pathogens and to diagnose bacterial plant diseases. The potentiality of the technique with plant pathogens has been mentioned by Coons and Kotila (2) and by Thomas (8, 11, 12) who also successfully identified species and strains of bacterial plant pathogens by this method (2, 6, 7, 9, 10). The two bacterial plant pathogens *Xanthomonas pruni* (E. F. Smith) Dowson and *Xanthomonas lactucae-scariolae* (Thornberry and Anderson) Burkholder which have been distinguishable only by pathogenicity (13) now may be distinguished by this technique (14, 15) when *X. pruni* bacteriophage (1) is used. With a sizeable volume of stored sterile *X. pruni* bacteriophage (15) and a large available collection of Xanthomonas species and cultures[4] used in extensive studies on the serological characterization of these organisms (3, 4, 5), a cooperative test on the lytic power of *X. pruni* bacteriophage on these bacteria was undertaken. The phage and directions for the technique were supplied by the senior author. The procedures were carried out and the results supplied by the junior authors.

The procedure used for the technique is as follows:

1. Prepare three tubes of young cultures showing turbidity upon agitation (24 to 48 hours' growth in 5-ml. broth tubes at pH 7.2 to 7.4 containing 0.2 per cent glucose—low glucose prevents the production of sufficient acid to interfere with lysis).

2. Inoculate two young culture tubes each with 0.5 ml. of phage inoculum and agitate for mixing, leaving one noninoculated young culture tube for control.

3. Incubate for 12 to 24 hours at 22°–24° C.

4. Observe relative turbidity of the agitated tubes at intervals. Lysis is demonstrated by relative decrease in turbidity when agitated.

The results show that *Xanthomonas pruni* bacteriophage causes a lysis in the six isolates of *X. pruni* (pruni 1, pruni 2, XP9, XP11, XP13, XP27)

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[4] The cooperation of Doctors Walter H. Burkholder, Mortimer P. Starr, Jack Wallin, and W. A. F. Hagborg in supplying many of the original cultures (3) is acknowledged.

but no definite reaction in the other species and their isolates. One culture of *X. manihotis* (XM12) and one of *X. phaseoli* (XP14) showed a questionable reaction which may have been due to partial inhibition of growth by the filterable non-phage products of lysed *X. pruni* cells in the impure phage inoculum. However, no evidence of lysis was obtained with other cultures of these two isolates. Lysis was nil for all other tested isolates of *X. manihotis* (XM6S, XM11) and other species and their isolates (*X. barbareae* XB1 and XB2; *X. begoniae* XB3, XB8M, and XB8S; *X. beticola* XB4; *X. campestris* XC2, XC3, XC13, and XC15; *X. campestris* var. *armoraciae* Armoraciae 1; *X. corylina* XC5, XC8M, and XC12R; *X. cucurbitae* XC6; *X. geranii* XG4 and XG6; *X. holcicola* XH3; *X. juglandis* XJ1, XJ3, XJ4, XJ5, and XJ5S; *X. lespedezae* XL1, XL2M, and XL2S; *X. malvacearum* XM2M and XM13; *X. papavericola* XP5, XP17; *X. pelargonii* XP15 and XP7; *X. phaseoli* var. *fuscans* XP26; *X. phaseoli* var. *sojense* XP21; *X. rubrilineans* XR1 and XR2; *X. taraxaci* XT11; *X. translucens* and its forms XT1, XT4, XT7, XT13, XT15, XT16, and 3061; *X. vesicatoria* XV4, XV8, and XV21; *X. vesicatoria* var. *raphani* XV16; *X. vignicola* XV18, XV18S, and XV20; and *X. vitians* XV2). The results presented do not exclude the possibility that the isolates of resistant species of the xanthomonads tested were resistant as a result of earlier infection by this or by some other closely related strain of bacteriophage. However, this appears unlikely since all *X. pruni* isolates tested responded with lysis.

The lysis of bacteria by this strain of *Xanthomonas pruni* bacteriophage is specific. If there exist other strains of *X. pruni* bacteriophage with nonspecific lytic capacity or other bacteriophages and strains that lyse *X. pruni* as well as other species of bacteria, such bacteriophages and strains would not be useful for the purpose of simple direct identification of bacteria but might be utilized for identifications through comparative lysis. *Xanthomonas pruni* bacteriophage is apparently more specific than are the cross-agglutination tests. There was no evidence of lysis with *X. corylina*, *X. phaseoli* v. *sojense*, and *X. lespedezae*, all of which made a compact serological group with *X. pruni* (3).

The phage-lysis method has promise as a general identification procedure if highly specific phages for additional or all pathogens can be found and preserved. The lysis technique, once the phage is prepared, is much simpler and faster than the usual staining, physiological, and serological procedures employed for identification. Whether the specificity of lysis parallels pathogenicity, genetic origin, any biochemical or serological property of any organism is unanswered to date but appears to be important. The success with the technique herein confirms previous claims.

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CHEMICAL SOIL TREATMENT FOR BLACK ROOT ROT OF TOBACCO IN THE GREENHOUSE

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(Accepted for publication October 28, 1948)

Tobacco growers almost universally practice some method of soil sterilization in preparing a plant bed area. The primary objective is the destruction of weed seeds, but the elimination of soil borne diseases also is important. Chemical methods of soil sterilization have come into increasing use and one of the newest chemicals is urea.[2] At rates of ½ to 1½ lb. per square yard of bed surface, various diseases, black root rot (*Thielaviopsis basicola* (Berk.) Ferraris),[3] root knot (*Heterodera marioni* (Cornu) Goodey),[4] and bacterial wilt (*Bacterium solanacearum* E. F. S.),[5] have been controlled. Results have been most satisfactory on the light sandy soils of the Southeast, and less so on the heavier soils where leaching is less rapid. It seems desirable to continue to search for more effective chemicals, particularly for use with heavy soils. The observations here reported included tests on the effectiveness of about 25 chemicals for use in soil sterilization against Thielaviopsis root rot of tobacco. The work was conducted during the winters of 1943 to 1947, inclusive.

One-gallon crocks with four seedlings of Maryland Broadleaf tobacco per crock were used in triplicate for each test. Each crock, except those of the healthy control, was filled with a half and half mixture of infested and sterilized soil into which the indicated chemical had been thoroughly incorporated. The treated soil was then held for a month or more in a moist condition before the tobacco seedlings were transplanted. The seedlings were lifted and their roots examined after approximately six weeks. Usually the effectiveness of the chemicals was further tested by planting and examining a second crop of seedlings. Between crops the soil was leached by several thorough waterings. Temperatures in the greenhouse were 50°–60° F. during the night; and 65°–75° F. during the day. Initial toxicity of the chemical to the plants would be a major factor with the first crop. The second crop would indicate the ease with which leaching might eliminate toxic chemical residues. Compounds were tried initially at the rate of 12 gm. per crock (1 lb. per sq. yd. of soil), and decreased in

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[2] Obtained as the commercial product Uramon.

[3] Henderson, R. G. Treatment of tobacco plant bed soil with nitrogenous fertilizers. Agr. News Letter (Du Pont) 9: 72–78. 1941.

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quantity in subsequent trials until decisive results were obtained. Tests with the more promising compounds were repeated.

Of the compounds investigated urea, sodium azide, hexamethylenetetramine, and sodium nitrite appeared most promising (Table 1). The compounds found to be unsatisfactory for partial soil sterilization against Thielaviopsis root-rot were calcium cyanamide, sodium formaldehyde sulfoxylate, hydrazine sulfate, hydroxylamine sulfate, 2,4-dinitro-diphenylamine, dicyandiamide, dipicrylamine, 2,4 diaminodiphenylamine, 4,4-diaminodiphenylamine, p,p-diamino diphenylmethane, s-diphenylthiourea, di-(B-hydroxyethyl) aniline, sodium salicylanilide, thymol, methyl violet, nile blue, malachite green, chloramin T, potassium ferrocyanide, and po-

TABLE 1.—*Control of black root rot (Thielaviopsis basicola) of tobacco by chemical treatment*

Chemical	Grams per Crock[a]	First Crop Plants		Second Crop Plants	
		Diseased	Healthy	Diseased	Healthy
		Number	*Number*	*Number*	*Number*
Check (Inoculated, not treated)	.....	12	0	12	0
Check, Healthy	.....	0	12	0	12
Urea	12	0	9[b]	3	7
Do	6	2	8[c]	1	11
Hexamethylenetetramine	12	0	0	0	11
Do	9	3	9[c]	4	8
Do	6	0	4	4	4
Sodium azide	1.0	0	0	0	12[b]
Do	0.8	0	12[b]	.....	.....
Do	0.6	0	12[b]	.....	.....
Do	0.5	0	10[b]	4	8
Sodium nitrite	12	0	6[c]	0	5
Do	6	4	8[c]	.....	.....

[a] Number of pounds to be applied to a square yard of bed surface is equivalent to $^1/_{12}$ number of grams per crock.
[b] Growth of tobacco greatly retarded as a result of chemical toxicity.
[c] Growth of tobacco slightly retarded as a result of chemical toxicity.

tassium ferricyanide. The last 2 compounds were much more injurious to tobacco than to the fungus; stunting of the seedlings and mottling of the leaves occurred with as little as 1.5 gm. per crock, and the injury persisted after leaching.

Urea at 12 gm. per crock (1 lb. per sq. yd.) and frequently at 6 gm., gave satisfactory protection with first crop seedlings. The plants were usually slightly stunted as compared to the healthy controls, and some were killed. Light infection of some of the roots of the second planting usually could be found.

Sodium nitrite appeared to be more effective than urea on replanting, but was rather toxic to the seedlings unless leached from the soil.

Hexamethylenetetramine gave some control at 9 gm. per crock in both tests. It was more toxic than urea to tobacco and adequate leaching is desirable. Stunting or killing of seedlings was evident with first crop

seedlings. This compound is used in human medicine and is relatively non-toxic to animals.

Sodium azide proved most effective of all the chemicals tested. Quantities of 0.5 to 1.0 gm. per crock gave good root-rot control with both crops of seedlings. Without leaching, the first crop of plants was killed; with leaching, 1.0 gm. per crock gave excellent disease control, but growth of tobacco was retarded. This compound is very poisonous to animals and it would be inadvisable to recommend its use until the hazards have been eliminated. It forms explosive compounds with metals such as copper.

Urea is the least expensive of the effective compounds, and it also has some value as a source of nitrogen. An attempt was made, therefore, to improve the effectiveness of urea through admixture of other compounds, particularly sodium azide, hexamethylenetetramine, and dichloramin T. No synergistic effects could be detected.

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SCLEROTINIA TRIFOLIORUM, A PATHOGEN OF LADINO CLOVER[1]

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(Accepted for publication November 11, 1948)

INTRODUCTION

Winter-survival is one of the major problems confronting growers of the Ladino variety of clover (*Trifolium repens* L.). Damage to old plants is usually most evident in the spring and is frequently attributed to winter-injury. Observations indicate the probable involvement of a number of factors including a destructive disease caused by *Sclerotinia trifoliorum* Erikss.

In northern Europe, *Sclerotinia trifoliorum* has long been recognized as an important pathogen of forage legumes. *Trifolium repens*, however, is usually considered resistant. Pape (5), failed to observe *Sclerotinia* attacking white clover, but he cites several authors who observed minor attacks on this host. Frandsen (2), inoculated *T. repens* with *Sclerotinia trifoliorum* and found it was not infected so readily as other species. In Kentucky, Gilbert and Bennett (3), observed *Sclerotinia* attacking white clover, but found other forage legumes more susceptible.

During the spring of 1945 and again in 1946 extensive killing of Ladino clover stolons was observed in nursery and field plots at State College, Pennsylvania. Many of the stolons were affected by a watery soft rot that left 4 or 5 cm. of tissue collapsed and flaccid. Other stolons in more exposed places were dead and disintegrating as though attacked earlier. In some cases, white masses of mycelium grew on the surface of diseased stolons and later formed sclerotia. Most sclerotia matured on the outside of affected stolons although some were found inside. These usually ruptured the stolon as they enlarged and were left in the soil when the stolon disintegrated. Apothecia emerging from a sclerotium imbedded in a stolon of Ladino clover are shown in figure 1, A.

Sclerotia were black, varied from 2 to 10 mm. in diameter, and were either simple or compound. Apothecia were light brown, the disk rested on the surface of the ground or was raised a few millimeters on a stipe. Stipes supporting the apothecia were 3 to 20 mm. long depending on the depth at which sclerotia were buried. Apothecia were collected from October until snowfall—only rarely were they found in the spring. Typical apothecia growing from sclerotia are in figure 1, B. On the basis of size and morphology of sclerotia and apothecia, cultural characteristics, and pathogenicity to Ladino clover and other legumes, the organism was identified as *Sclerotinia trifoliorum*.

[1] Contribution No. 89 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Penna., in cooperation with the Northeastern States.

[2] Associate Pathologist.

The present study was undertaken because of the potential destructiveness of the disease to a valuable forage crop.

MATERIALS AND METHODS

Cultures of the organism were obtained from sclerotia and ascospores. Sclerotia were surface sterilized, halved aseptically, and plated on Difco potato-dextrose agar in Petri dishes.

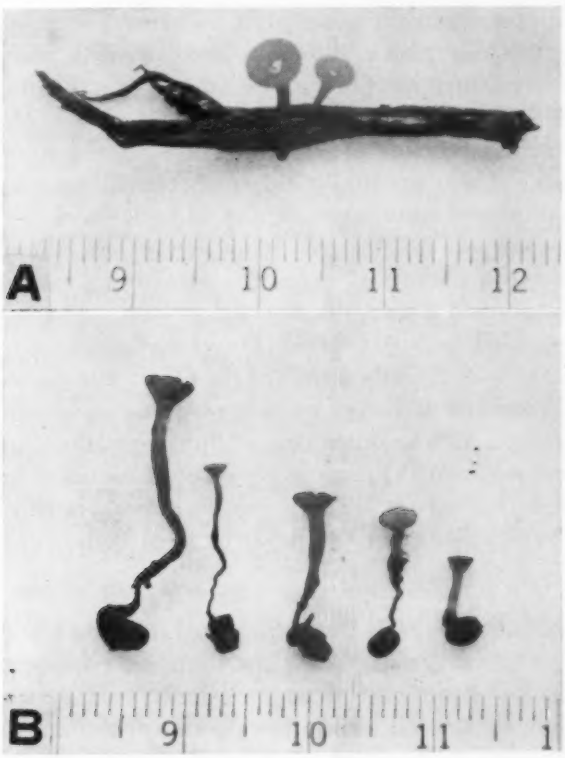

FIG. 1. A. Apothecia emerging from a sclerotium imbedded in part of a dead and disintegrating stolon of Ladino clover. B. Apothecia with stipes of different lengths. A millimeter scale is shown in both photographs.

Monoascospore cultures were obtained by a modification of Tapke's method (6). Instead of placing apothecia in small vials of alcohol as described by Tapke, an apothecium was held with a forceps just below the disk and a drop of 95 per cent ethyl alcohol was applied to the base of the stipe. By holding the discharging apothecium inside a partially covered Petri dish of agar while slowly rotating the dish, a fairly uniform distribution of ascospores was achieved. Individual spores were located with the high power lens of a dissecting microscope and transferred to a Petri dish of agar so marked that each spore could be readily located and observed for germination. If the spore germinated and was free of contaminants, it was trans-

ferred to a fresh tube of potato-dextrose agar. In this way, more than 100 monoascospore cultures were isolated from apothecia collected in stands of Ladino and red clover.

Infection tests were conducted with cultures obtained from sclerotia and from ascospores. Hosts inoculated included Ladino clover, red clover, other species of *Trifolium, Medicago, Melilotus,* and *Lotus.*

CULTURAL CHARACTERISTICS OF ISOLATES

On Difco potato-dextrose agar, most cultures produced a white, appressed to fluffy colony with regular margins. Growth was usually rapid, the mycelium frequently reaching the periphery of a 90-mm. Petri dish in 5 days at 20° C. Most cultures produced sclerotia after the hyphae reached the edge of the dish. Sclerotia were first noticeable as clusters of dense, white mycelium. These gradually enlarged, turned gray and then black. Most cultures produced mature sclerotia in 14 to 21 days.

Some monoascospore isolates grew more slowly than others. These either eventually produced clumps of mycelium resembling partially developed sclerotia or maintained a uniform mycelial growth. The majority of cultures were classified into 4 types: (1) closely appressed mycelium, no sclerotia (2) appressed mycelium toward the center but the outer one-fourth of the colony composed of fluffy, white mycelium; no sclerotia (3) mostly appressed mycelium with an outer ring of fluffy mycelium containing a few large, compound sclerotia (4) same as type 3 but with many sclerotia around the outer edge of the colony. There was no noticeable cultural difference between isolates obtained from red clover or from Ladino clover.

INFECTION TECHNIC

Preliminary infection tests were conducted on mass seedings of Ladino clover in flats of steamed soil. After the seedlings reached the 2 or 3 leaf stage and formed a solid cover, they were inoculated by scattering pieces of agar inoculum on the soil among the plants. The flats were then moistened, covered with a double layer of cheesecloth and incubated on a greenhouse bench at 18°–20° C. This method resulted in death of seedlings in patches where they had been exposed, but was found unsatisfactory because much of the agar inoculum was rapidly overgrown by contaminants that reduced its effectiveness. To overcome this difficulty, cultures were grown for 21 days on a medium composed of 2 parts wheat, 1 part oats (5). This time was ordinarily required for the organism to spread through the grain and begin to form sclerotia. The effectiveness of the fresh grain inoculum was tested on crowns or stolons of potted plants under high humidity and at 18°–20° C. Good infection was obtained.

EFFECT OF SCLEROTINIA ON INOCULATED LADINO CLOVER

When fresh grain inoculum was spread along a row of Ladino clover seedlings and the inoculated plants were kept moist and at 18°–20° C., they

became infected in 3 or 4 days. Earliest visible symptoms consisted of drooping and wilting of leaves and petioles. Damaged leaves usually turned gray-green as though scalded and the infected seedlings became flaccid and collapsed shortly thereafter. Most seedlings were dead 7–10 days following inoculation.

Similarly, when grain inoculum was applied to the crowns or stolons of older plants growing in pots they were attacked as readily as seedlings but it took longer to kill them. Younger leaves and petioles were infected and collapsed first; stolon tips and lateral buds were attacked next. As the mycelium overgrew other parts of the plant, older leaves and stolon internodes were damaged or killed. Stolons infected under controlled conditions became flaccid and watersoaked like those naturally infected in the field. When handled, the stolons were readily pulled apart.

PATHOGENICITY OF MONOASCOSPORE ISOLATES TO LADINO AND RED CLOVER

Preliminary tests showed that cultures of *Sclerotinia trifoliorum* obtained from sclerotia were pathogenic to seedlings and mature plants of Ladino clover. Additional tests were therefore conducted to determine the pathogenicity of monoascospore isolates. Björling (1), Frandsen (2), and others emphasized that a sclerotium may be comprised of the mycelium of one or more biotypes of *Sclerotina*. Observation of anastomoses between hyphae indicates that heterocaryotic mycelium may develop and form sclerotia. Cultures derived from such sclerotia would therefore not represent homozygous lines of the organism. For the pathogenicity trials, 28 monoascospore cultures representing isolates from 13 apothecia and from each of the 4 cultural types were selected. After each isolate was cultured on the wheat-oats medium fresh inoculum from it was placed in contact with crowns of seedlings and older plants of Ladino clover and mature plants of red clover. In the tests with Ladino clover, 4 seedlings and 6 mature plants were inoculated with each isolate of *Sclerotinia*. In the trial with red clover, 12 mature plants were inoculated with each isolate. The inoculated plants were thoroughly watered, placed at random and incubated in a large moist chamber (4) in a greenhouse at 18°–20° C. During late spring when greenhouses became too warm, inoculated plants were incubated in a constant temperature cold room at 15° C. Plants incubated in the cold room received illumination from 150-watt Mazda lamps 12 hours each day. Depending upon position, each plant received 50–400 foot candles of light as measured by a Weston photometer.

Most monoascospore isolates from Ladino or red clover were equally pathogenic to each host. With few exceptions, seedlings of Ladino clover were more susceptible than older plants. There was some indication of differences in virulence among isolates but striking differences were probably masked because of variation in physiologic resistance among plants of each host tested. Similarly, it was not possible to distinguish slight variations in pathogenicity among monoascospore cultures from individual apothecia.

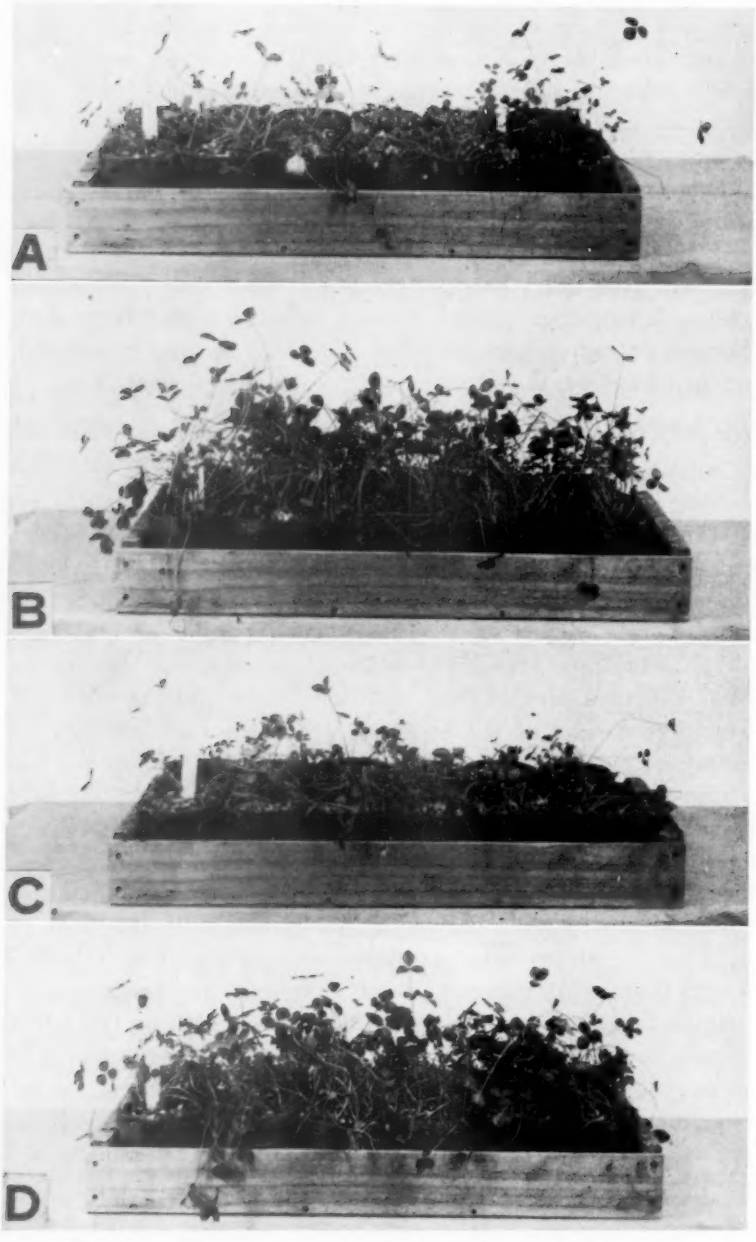

FIG. 2. Flats of 18 representative pots of Ladino clover inoculated with (A) pathogenic isolate R-1-4 from red clover, (B) less virulent isolate R-3-8 from red clover, (C) pathogenic isolate L-6-8 from Ladino clover, and (D) less virulent isolate L-1-10 from Ladino clover.

Since differences in pathogenicity of monoascospore isolates were indicated, larger numbers of plants were inoculated in a second test.

The plants were grown in 3-inch pots in a greenhouse and the stolons were clipped when they reached the edge of the pot. Four monoascospore isolates were selected: one pathogenic isolate (L-6-8) from Ladino clover and one (R-1-4) from red clover, and a less virulent isolate from each host (L-1-10 and R-3-8). Each isolate was grown in flasks of wheat-oats mixture and the inoculum was scattered over stolons of each plant. Care was taken to inoculate each plant with approximately the same amount of inoculum. Controls were not inoculated. The plants were then watered and incubated in a cold room at 15° C. The plants received illumination from 150-watt Mazda lamps 12 hours each day.

Striking differences in pathogenicity among the selected isolates (Table 1) were readily detected when the larger numbers of plants were used. Though there undoubtedly were differences in susceptibility among plants

TABLE 1.—*Comparative virulence of four isolates of Sclerotinia trifoliorum to mature plants of Ladino clover*

Infection Class	Number of plants in each infection class after inoculation with isolate				
	R-1-4	L-6-8	R-3-8	L-1-10	Control
Slight	5	9	41	23	0
Slight +	0	1	14	16	0
Moderate	12	8	14	15	0
Moderate +	11	14	2	4	0
Severe	24	21	1	2	0
Severe +	17	10	0	0	0
Dead	3	1	0	0	0
No injury	0	0	0	0	18
Totals	72	64	72	60	18

within each group inoculated, the overall damage caused by each virulent isolate was greater than that of its less virulent counterpart. This confirmed the observation of differences in virulence among monoascospore isolates tested earlier. The use of clones would perhaps reveal more subtle differences in pathogenicity and would reduce the number of plants needed for a test. The effect of pathogenic and less pathogenic isolates on plants of Ladino clover is shown in figure 2.

PATHOGENICITY TO OTHER SPECIES OF LEGUMES

Both Pape (5) and Frandsen (2) published reviews of literature concerning the host range of *Sclerotinia trifoliorum*. According to Frandsen (2) more than 80 species in 15 families are attacked by *Sclerotinia*. However, races did not appear to be restricted to certain host species since cultures from different legumes were no more infective to individuals of a selected host spectrum than were isolates from one host. Since *Trifolium repens* was listed as a species ordinarily resisting infection, it seemed desirable to test isolates from Ladino clover for their pathogenicity to other species of *Trifolium*, *Medicago*, *Melilotus*, and *Lotus*.

Three pots of seedlings and four or five mature plants of each species were inoculated. Seedlings were 6 to 10 weeks old while mature plants were either flowering or were in bud and in some cases required clipping before they were inoculated. Inoculum consisted of a composite sample of 7 pathogenic monoascospore isolates grown on the wheat-oats medium. The inoculum was distributed at the base of seedlings and around the crown of older plants; controls were not inoculated. All plants were incubated at 18°–20° C. in a greenhouse. Notes on infection were taken at intervals from 7 to 15 days following inoculation.

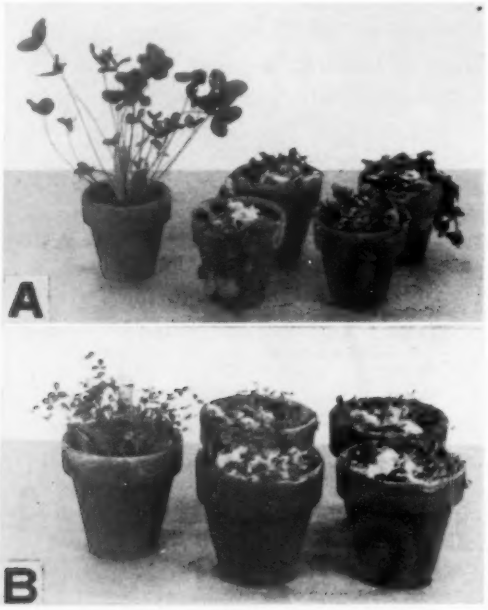

FIG. 3. A. *Trifolium hybridum* killed by *Sclerotinia trifoliorum*. B. *Trifolium glomeratum* severely damaged by *Sclerotinia*. In each photograph a noninoculated control is at the left.

Most species tested were susceptible to the inoculum used (Table 2). Several species of *Trifolium*, *Medicago*, and *Melilotus* not previously tested were infected. At least 3 species, *Trifolium resupinatum*, *T. fragiferum*, and *T. dubium*, were not damaged so severely as the others. *T. dubium*, in particular, appeared moderately resistant to infection with the isolates used. This suggests possible sources of resistance to the disease in species of the genus *Trifolium*. There was no appreciable difference between seedling and mature plant reaction. Occasionally differences occurred, but in most cases, these could be attributed to drying or contamination of inoculum before the organism became established on a host. The severity with which *Sclerotinia trifoliorum* attacked some species of *Trifolium* is illustrated in figure 3.

TABLE 2.—*Susceptibility of seedlings and older plants of legume species to artificial inoculation with Sclerotinia trifoliorum*

Species	Infection[a] on seedlings	Infection[a] on older plants
Trifolium repens (Ladino)	S+	S–M*
T. repens (White Dutch)	M	M
T. pratense	S+	S
T. fragiferum	M	Sl
T. hirtum	S	M*
T. carolinianum	S+	–*
T. incarnatum	S+	S–
T. resupinatum	Sl–	Sl*
T. subterraneum	S	–
T. striatum	S+	M*
T. glomeratum	Sl–	S+*
T. arvense	Sl–	S–M*
T. procumbens	Sl	S*
T. dubium	Sl	Sl+*
T. lappaceum	S+	S+*
T. agrarium	S–	S*
T. reflexum	S+	–*
T. cernuum	M+	S+*
T. hybridum	–	S+
T. alexandrinum	S+	S+
Medicago arabica	S+	S–M*
M. obscura	Sl–	S*
M. lupulina	Sl+	S+
M. hispida	S+	Sl
M. minima	M	M*
M. sativa	–	S+
Melilotus alba	S+	S+
M. indica	S+	S–*
M. suaveolens	S	S+*
M. officinalis	S+	S+
Lotus corniculatus	Sl–	S

[a] Sl = slight infection.
M = moderate infection.
S = severe infection.
(+) = greater infection; (–) = less infection.
* Denotes species not previously listed as susceptible to *Sclerotinia trifoliorum*.

DISCUSSION

Ladino clover is rapidly becoming one of the most important pasture plants in areas of the United States where it is adapted. As it becomes more widely grown, diseases affecting it appear more frequently and with greater destructiveness, particularly in localities where large acreages are found. *Sclerotinia trifoliorum* is one of the pathogens potentially capable of causing severe damage to a stand. Because the fungus attacks stolons at low temperatures, much of the damage observed in early spring may be attributed to winter-injury.

Observations indicate *Sclerotinia* is probably not the sole cause of winter-injury to this crop. If conditions are favorable, however, there is every indication that the disease may play a major role in loss of stands.

Results of studies with red clover by European workers indicate that the disease can probably be best controlled by breeding resistant varieties.

Should adequate sources of resistance not be found in *Trifolium repens*, there is evidence that some measure of resistance exists among other species of *Trifolium*. This might prove useful if species hybridization in the genus *Trifolium* can be accomplished.

SUMMARY

Sclerotinia trifoliorum was observed attacking Ladino clover.

Monoascospore isolates yielded four cultural types that varied from strictly mycelial types to forms that produced numerous sclerotia. Pathogenicity of monoascospore isolates was established on Ladino and red clover. Differences in pathogenicity of several monoascospore cultures were determined with a number of plants of Ladino clover.

Infected stolons of Ladino clover became flaccid and watersoaked, and death resulted after a severe attack. Seedlings collapsed and died 7 to 10 days following inoculation.

Though a composite sample of the organism was pathogenic to a number of species of *Trifolium*, *Medicago*, and *Melilotus*, several species of *Trifolium* resisted infection.

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PYTHIUM ROOT ROT OF AROIDS AND EASTER LILIES

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(Accepted for publication November 11, 1948)

Chinese Evergreen (*Aglaonema simplex* Blume) became a commercial crop in Florida about 1934, at which time there were about two acres planted in the State. During the following eight years there was an increase to approximately 15 acres. This in turn has been followed by rapid decline due to Pythium root-rot.

During the period of expansion, Chinese Evergreen was produced in outdoor beds under slat sheds, which involves a high overhead investment. For such an investment to yield profitable returns, it was necessary in the early years to grow successive crops of the Chinese Evergreen. As demand warranted, other ornamentals, mostly aroids, were produced under the sheds, in rotation with Chinese Evergreen. The soils used for these crops were mostly low and sandy, but they were maintained in a high state of fertility by the addition of muck, compost, and commercial fertilizers, and a mulch of leaves or other vegetable matter was placed on the soil surface after the plants were set.

Prior to the war, most of the propagating material was imported from China. The imported canes were cut into sections bearing one or two "eyes" and started in greenhouses or planted directly in the beds under the sheds. This was usually done in April or May, and some of the resulting plants were marketed by the end of the same year. During the war when the canes could not be imported, growers began to propagate the plants by bud cuttings. This method of propagation produces marketable plants in a shorter period than propagation by cane cuttings, but it does not reduce the loss from the root-rot to any appreciable extent.

The first trouble with Pythium root-rot of Chinese Evergreen in Orange County was reported in 1943.[1] Since that time the disease has been observed in Chinese Evergreen, other aroids, and Easter lily in several localities of the State. When the first inspection of nurseries was made in Orange County, affected plants occurred in areas of various sizes, but within a year or two the entire area in some nurseries had become involved.

First sign of the trouble on above-ground parts of the plants is stunting. This is followed by drooping of the leaves, yellowing, and death of the lowest ones. Finally, some of the stems rot off at the ground level and the plants drop over, but decay of the stems apparently does not continue. Early symptoms of the disease on the roots are manifest as light-brown, water-soaked lesions of various lengths, occurring on any part of the root. In later stages the diseased tissue turns brown and disintegrates so that most of the diseased roots remain in the soil when the plants are

[1] Tisdale, W. B. Root rot of aroids. U. S. Dept. Agr., Plant Dis. Rptr. 27: 306–308. 1943.

removed. In the final stages all roots have rotted and nearly all leaves up to the bud are dead. Many mulched plants develop roots above the soil level and these become infected on coming in contact with the soil.

Several other aroids planted under sheds with Chinese Evergreens had similar disease symptoms on the roots, but in no case was the damage as severe as that in Chinese Evergreen. The plants affected were *Dieffenbachia picta* Schott., *Nephthytis Afzelii* Schott., *Philodendron cordatum* Kunth., and *Scindapsus aureus* Engler.

In 1944 a grower in Dade County reported a serious condition of Easter lilies in his planting. He had noticed the disease in milder form the previous year, and bulbs from that crop were planted on the same land, and on additional acreage in 1944. The soil was of the Rockdale series, and very different from that in which the Chinese Evergreen and other aroids are produced. When inspected in March of that year, practically every plant in fields planted to Easter lilies the previous year showed advanced stages of the disease, and the condition of plants in other fields from the same lot of bulbs was little better. The plants were severely stunted, the lowest leaves were dead, and the other leaves were yellow with dead tips. Some plants were dead except for a few bud leaves. This condition may have been caused in part by virus diseases, but the plants were in such advanced stages of decline that diagnosis was impossible.

Root decay was extensive in all plants examined, and many roots were dead. New roots had developed on some stems at about the ground level and these had dead tips or brown lesions on other parts. Also, the outer scales of many bulbs were dead or showed brown lesions of various sizes. Some bulbs of the 1944 crop were saved and planted later in the year, and they failed completely. Since 1945, commercial growing of Easter lilies on the Rockdale series of soils in Dade County has been practically discontinued because of the losses caused by Pythium root rot.

Several fungi were obtained consistently in cultures from all the host plants, but none of them, except *Pythium splendens* Braun,[2] produced any symptoms of disease in inoculation experiments. Cultures of the *Pythium* isolated from all the aroids listed above and Easter lily have produced a root-rot typical of that developing in them in the nurseries, and cultural characters and cross inoculations have shown that the causal organism is the same for all crops, and is widely distributed in Florida. *Pythium splendens* has been reported to attack a wide range of host plants,[3] but none of the ones specified in this article was included in the list.

When rooted cuttings of Chinese Evergreen and young Easter lily plants were set in artificially inoculated soil held at moderate temperatures, root lesions developed within 10 days and the root systems were almost completely destroyed in 21 days (Fig. 1 and 2). In all tests the disease developed most rapidly at moderate temperatures, and least rapidly at

[2] Determination made by John T. Middleton.

[3] Middleton, John T., The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club 20: 1–171. 1943.

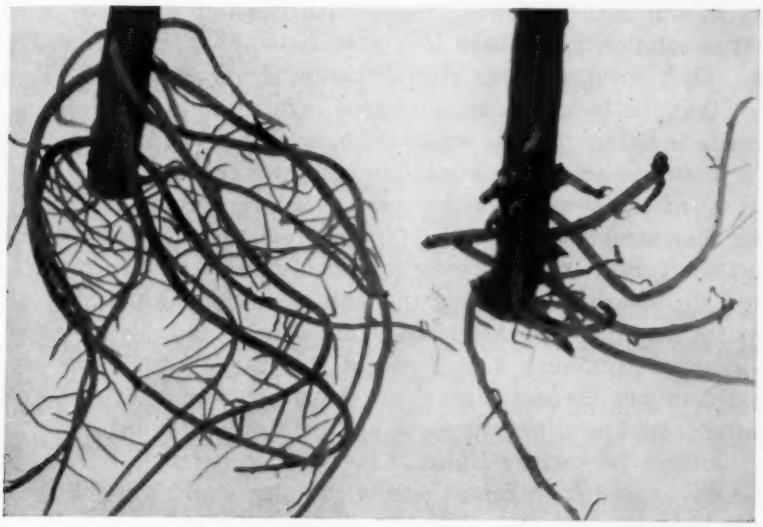

FIG. 1. Pythium root rot of Chinese Evergreen. Healthy plant at left set in sterilized soil and diseased plant after 21 days in soil artificially inoculated with *Pythium splendens.*

high temperatures. This coincides with observations made in the nurseries.

Activity of the fungus and its distribution in the nurseries suggests general sterilization of the soil for control. A preliminary test made in a

FIG. 2. Pythium root rot of Easter lily. Healthy plant at left set in sterilized soil and diseased plant (right) after 21 days in soil artificially inoculated with *Pythium splendens.*

nursery in which the soil was treated with one-half gallon of 1–50 formaldehyde solution per square foot gave about 85 per cent good plants that had only a few root lesions when they were of marketable size. However, the fact that the beds became reinfested before the plants reached marketable size indicates that the walkways between beds and bed wall boards, must be treated to prevent reinfestation in a short time.

Several of the new fungicides were tested in potted soil to ascertain whether they would eradicate the fungus from the soil without injuring the plants. Tersan (tetramethyl thiuram disulfide) and Fermate (ferric dimethyl dithiocarbamate) were the only materials tested that showed promise for this purpose. After the plants were set in infested soil enough of the solution containing 1 or 2 gm. of the chemical to 1 liter of water was added to wet the soil throughout the pot. Neither of these concentrations appeared to injure the roots, and both prevented infection. However, in outdoor tests where 1 pint of the solution per square foot was dug into the soil immediately before setting the plants and was followed with the same volume at 30-day intervals, applied on the soil surface with a sprinkling can, no appreciable benefit was obtained. Some growers have resorted to propagating Chinese Evergreen on benches in the greenhouse, using steam-sterilized soil. When clean planting stock is used, the plants have remained free of Pythium root-rot, but this has necessitated a great reduction in the number of plants propagated.

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PHYTOPATHOLOGICAL NOTES

The Rôle of Thiamin and Certain Related Compounds in Pigment Production by Corynebacterium michiganense (Erw. Smith) Jensen.—Variation in pigmentation has been described for a number of plant pathogenic as well as for many other bacterial species. Bryan,[1, 2] for example, has isolated white and pink variants of the usual yellow form of *Corynebacterium michiganense* (Erw. Smith) Jensen. More recently Ark[3] with the use of acenaphthene-saturated broth induced white mutants in this species. A correlation was found to exist[4] between pigmentation and virulence. The yellow form was found to be the most virulent for the tomato, while the pink form was far less pathogenic. Despite the fact that considerable work has been done on pigmentation, very little is known about the physiological mechanism involved in the production of these substances by bacteria.

The findings reported here are the result of observations made on two isolates of *Corynebacterium michiganense* that had been kept as stock cultures for a number of years. In their cultural, staining, and disease-producing properties both were typical of the species. It was observed that when these isolates were cultivated on certain media such as (Difco) dextrose agar growth was excellent at 25° C. but there was not the slightest indication of the presence of the characteristic yellow pigment. When the dextrose agar was supplemented, however, with one per cent to two per cent (Difco) yeast extract the yellow pigment was produced. These results demonstrated, therefore, that pigmentation in this instance was dependent upon some factor or factors present in the yeast extract. As a result of this finding certain vitamins of the "B" complex which included thiamin HCl, pyridoxine, niacin, riboflavin, Ca pantothenate, inositol, and para aminobenzoic acid were tested in concentrations ranging from 100 mgs. to 0.01 mg. per liter of media. All were sterilized by filtration. Of the substances tested only thiamin HCl was capable of replacing the yeast extract in the dextrose agar. Excellent yellow pigmentation was shown by the bacteria when concentrations of this compound ranging from 100 mg. to 1 mg. per liter of media were used. A cream colored growth was produced at a concentration of 0.1 mg. of thiamin HCl per liter. The thiazole (4-methyl-5-β-hydroxyethyl thiazole) portion of the thiamin molecule was capable of replacing the thiamin, while the pyrimidine fraction (2-methyl-5-ethoxymethyl-6-aminopyrimidine) was without effect. A sample of γhydroxypropyl thiazole, however, was inactive. Cocarboxylase, on the

[1] Bryan, Mary K. An albino strain of *Aplanobacter michiganense.* (Abstr.) Phytopath. **20**: 141. 1930.

[2] Bryan, Mary K. Color variations in *Aplanobacter michiganense.* Phytopath. **21**: 559. 1931.

[3] Ark, P. A. Mutation in certain phytopathogenic bacteria induced by acenaphthene. Jour. Bact. **51**: 699–701. 1946.

[4] Fawcett, Edna H., and Mary K. Bryan. Color in relation to virulence in *Aplanobacter michiganense.* Phytopath. **24**: 308–309. 1934.

other hand, was effective in the same concentrations as was thiamin and thiazole. A white mutant of this organism did not respond to thiamin. The physiological action of these compounds in pigment production is being investigated.—ARMIN C. BRAUN, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

A New Method of Evaluating Fungicides.—Most of the laboratory methods of evaluating fungicides are based on the ability of the fungicides

TABLE 1.—*Relation of concentration and exposure time to effectiveness of five fungicides against Fusarium oxysporum (Schl.) var. gladioli Massey*

Fungicide and concentration[a]	Exposure time and fungus growth[b]			
	15 min.	30 min.	1 hr.	2 hr.
Dow 9B (zinc trichlorophenate)				
1.00 per cent	−	−	−	−
0.50	+	+	−	−
0.25	+	+	+	−
Puraturf 177 (phenyl mercuri triethanol ammonium lactate)				
2.00 per cent	−	−	−	−
1.00	+	−	−	−
0.50	+	+	−	−
0.25	+	+	+	+
New Improved Ceresan (ethyl mercury phosphate)				
0.047 per cent	−	−	−	−
0.031	+	−	−	−
0.016	+	+	−	−
0.008	+	+	−	−
0.004	+	+	+	+
Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate)				
0.06 per cent	−	−	−	−
0.04	+	+	−	−
0.03	+	+	+	−
Mercuric chloride				
0.010 per cent	−	−	−	−
0.006	+	−	−	−
0.005	+	+	−	−
0.004	+	+	+	−

[a] Concentration was based on the commercial products and not on active ingredients.
[b] + indicates growth of the fungus; − indicates no growth.

to inhibit spore germination. Since none of the methods commonly employed is especially suited for use with Fusarium, the effectiveness of various fungicides for control of such diseases as Fusarium rot of gladiolus has been determined entirely by field tests. With the introduction of so many new fungicidal materials it is becoming increasingly difficult to test them on a field basis. A simple laboratory test has been developed to save time in screening fungicides effective against the gladiolus Fusaria.

Several sterile pieces of No. 8 cotton thread 3 to 4 inches long are laid

on the surface of potato-dextrose agar in a Petri dish (Fig. 1, A). The fungus is planted in the plate and allowed to grow until the entire surface of the agar is covered by the fungus. The pieces of thread are then removed from the culture plate with a sterile forceps and cut into pieces about $\frac{1}{8}$ inch long with sterile scissors. These small pieces are collected in

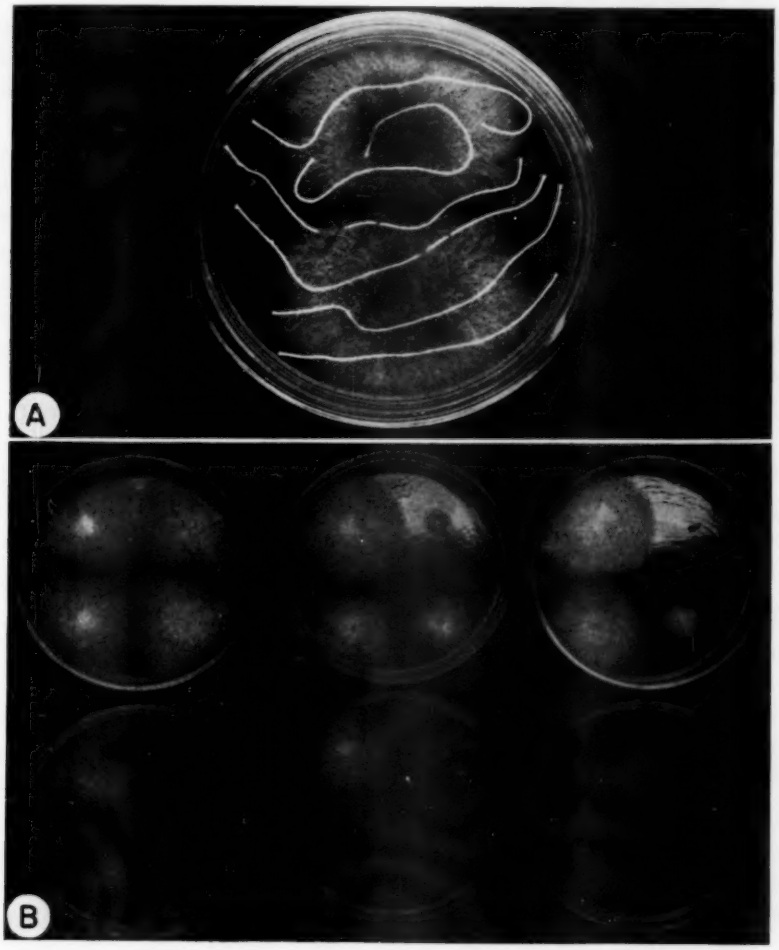

FIG. 1. A. Fusarium growing over cotton threads on surface of agar plate. B. Growth of Fusarium from treated threads 5 days after plating; left to right, upper row: water, Parzate, Puraturf 177; lower row: Dow 9B, Lysol, New Improved Ceresan. Starting at upper right sector in each plate and reading clockwise, treatments were 2 hours, 1 hour, 30 minutes, and 15 minutes.

a sterile Petri dish from which they are removed with a sterile forceps and placed in the fungicide preparation.

As a preliminary test, pieces of the fungus-infested thread are rolled in a small quantity of the dry fungicide until they are well coated and then planted in potato-dextrose agar plates. The plates are kept at room

temperature (approx. 22° C.) for 7 days and examined for growth of the fungus. Materials which do not completely inhibit growth of the fungus are not tested further.

Several concentrations of the effective materials are made up for further testing. The small pieces of fungus-infested thread are immersed in these prepared solutions and allowed to remain for predetermined lengths of time. They are then removed, touched momentarily to a piece of sterile filter paper to remove the excess solution, and planted in an agar plate. Each series is run in duplicate. The plates are kept at room temperature for 7 days and examined for growth of the fungus.

A sample of the results of one test in which this method was used is shown in table 1. These data show that two factors are involved in inhibiting growth of the fungus. They are concentration of the fungicide and length of exposure to the fungicide. Even though a material is not fungicidal when used at low concentrations or with short exposures, some fungistatic action is evident. As shown in figure 1, B, fungistatic action increases directly with the length of exposure until the fungicidal point is reached.

The method should prove useful in testing fungicides against any fungus that grows well on laboratory culture media. It saves time in determining which materials merit further trial on a field basis and at the same time gives some idea as to the concentrations and treating times that will be necessary to be effective.—J. L. FORSBERG, Section of Applied Botany and Plant Pathology, Illinois Natural History Survey, Urbana, Illinois.

Rhizoctonia Neck and Bulb Rot of Iris in the Pacific Northwest.[1]—For the past two years a neck and bulb rot of the Wedgewood variety of bulbous iris (*Iris tingitana* hybrid Boiss. & Reut.) has been observed in the Puyallup and Mt. Vernon bulb growing areas in Washington. This rot is present in addition to the more common crown rot caused by *Sclerotium delphinii* Welch and apparently has been confused with it. The symptoms of crown rot (*Sclerotium*) differ from the symptoms of the neck and bulb rot (*Rhizoctonia*). In crown rot, numerous small, dark reddish-brown sclerotia are associated with a rot produced on tissue at the base of the leaves and flower stalks. This crown rot causes the leaves to die back from the tips and the tops to fall over. In the case of the neck and bulb rot, however, a soft, light brown rot at the neck of the bulb results in the death of all above ground parts without any die-back of the leaf tips (Fig. 1). In severe cases of neck and bulb rot dark brown lesions occur on the outer scales, and the bulbs may be almost completely rotted away. These symptoms correspond to those reported as caused by *Rhizoctonia* on tulips by MacLean.[2] No sclerotia were found.

[1] Published as Scientific Paper No. 795, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Wash.

[2] MacLean, Neil Allan. Rhizoctonia rot of tulips in the Pacific Northwest. Phytopath. 38: 156–157. 1948.

FIG. 1. Symptoms of *Rhizoctonia solani* injury on Wedgewood iris. × ½.

Isolations made on 2 per cent potato-dextrose agar from decayed tissue developed a light brown, slow growing, mycelium with the typical right-angled branching and constrictions of *Rhizoctonia*. Hyphal strands measured 5.5–8 μ in diameter. The causal agent is believed to be *Rhizoctonia solani* Kuehn.—CLARENCE H. SONDERMAN and NEIL ALLAN MACLEAN, State College of Washington, Pullman, Wash.